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with some Notes on Hormone-Gold and Hormone-Salazopyrin Therapy.

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PREVENTION OF SEVERE CARDIOVASCULAR AND SMOOTH MUSCLE NECROSIS IN THE RAT BY THYRO-PARATHYROIDECTOMY[1]

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IN THE course of studies on experimental arteriosclerosis in the albino rat (1, 2 and 3), it was noted that inhibition of thyroid hormone production by propyl thiouracil (4) had a protective effect against the development of cardiovascular and smooth muscle necrosis as well as against secondary calcification following standard renal injury. The parathyroid hormone likewise was believed involved in the production of these lesions since marked enlargement of the parathyroid glands was one of the alterations encountered most consistently (5 and 6), and since secondary hyperparathyroidism resulting from renal impairment has long been known to cause vascular damage and metastatic calcification (7).

It seemed of particular interest, therefore, to investigate the effect of surgical removal of the thyroid and parathyroid glands in the albino rat upon the development of the typical pathologic-anatomical picture induced by our method. In the present paper it will be demonstrated that thyro-parathyroidectomy provides clear-cut protection against the emergence of muscular necrosis and its sequelae.

MATERIALS AND METHODS

Closely bred, 6–9 months old, male and female albino rats from our own colony maintained on Purina chow and tap water ad libitum, were housed in individual metabolism cages throughout the experimental period.

Thyro-parathyroidectomy was performed under ether anesthesia with the aid of a binocular loupe. No serious postoperative complications were encountered. Four weeks were allowed to elapse between the surgical procedure and use of these rats.

Standardized renal damage was induced in intact and thyroparathyroidectomized animals with a method previously outlined in detail (2). In brief, the procedure entails

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[1] This work was carried out with the aid of grant No. H-890 (C4) from the National Heart Institute of the National Institutes of Health, U.S. Public Health Service.

the intraperitoneal injection of a single excessive dose of sodium acetyl sulfathiazole (0.5 gm./kg. body weight). Renal excretion of poorly soluble acetyl sulfathiazole causes temporary obstruction to the urinary flow (renal blockage) due to massive deposition of crystals in the tubules of the kidney. The resulting severe injury to the tubular epithelium initiates, in turn, a series of characteristic organic and functional changes in the kidney frequently referred to under the term "obstructive nephropathy."

In intact rats, the renal injury is followed almost invariably by severe disseminated necrosis in the cardiovascular system; similar lesions also appear frequently in the gastrointestinal tract. These alterations, conspicuous to the naked eye, develop within a period of 5 to 7 days, and are usually accompanied by a phase of marked hypertension. There is extensive secondary calcium imbibition of necrotic areas. Pronounced enlargement of the parathyroid and adrenal glands and thymic atrophy invariably precede these severe changes.

Serial microdeterminations of calcium (8), inorganic phosphate (9), cholesterol (10), and total carbon dioxide content (11), as well as terminal estimations of the non-protein nitrogen level (12), were performed on the blood plasma of each rat. Tail blood served for serial determinations, whereas neckblood obtained at sacrifice was used for terminal assays. The blood pressure was measured at predetermined intervals with the aid of a photoelectric tensometer using the hind paw of the unanesthetized rat (13). The average of at least three satisfactory consecutive readings was considered to represent the systolic blood pressure.

Complete postmortem examination was performed on every animal. The wet organ weight of the heart, thymus gland, adrenals and kidneys was determined to the nearest milligram on a torsion balance immediately upon removal. All important organs were fixed in formalin and sectioned for microscopic study using routine and special stains.

RESULTS

a) *Pathologic-anatomical findings*

Table 1 contains a compilation of pathologic-anatomical findings and of changes in body weight and organ weights of individual rats from a representative experiment with 38 male animals. Of these 22 rats had been thyro-parathyroidectomized (TPX), whereas the remaining 16 had been left intact.

It is apparent that 11 intact rats (Nos. 6 to 16) and 13 TPX animals (Nos. 24 to 38) survived the standard renal injury for at least one week. All of these, except for 2 TPX animals, developed severe obstructive nephropathy of similar extent. However, completely thyro-parathyroidectomized rats showed no trace of nephrocalcinosis, whereas calcium deposition in necrotic tubules (calcifying nephrosis) was the rule in *intact* animals.

Despite the otherwise close similarity of the renal injury, TPX rats manifested no lesions in the arterial tree, the stomach and myocardium, except for animal No. 25, which revealed some foci of necrosis and infiltrations with mononuclear cells in the myocardium, and animal No. 37 which had myocarditis only. Intact rats, on the other hand, showed the usual high incidence of severe lesions in all three of the above-named locations. The macroscopic picture of typical lesions in the cardiovascular system and gastrointestinal tract is exemplified in a series of photographs (Fig. 1).

TABLE 1. THE INFLUENCE OF THYRO-PARATHYROIDECTOMY (TPX) UPON MUSCULAR NECROSIS IN THE RAT

Rat No.	*Body weight in gm.* Pre-TPX	Pre-block	Lowest post-block	Final	Death, days post-block	Wet organ weight in mg. Heart	Thymus	Adrn.	Kidneys	*Gross and microscop. patholog* Heart	Coron. art.	Aorta	Stomach	Kidney
						Intact animals								
1	292	310			<2	1025	105	50	2685	−	−	−	−	−
2	341	357			<2	1297	76	65	3360	−	−	−	−	±
3	296	292			>2	850	130	58	2175	−	−	−	−	±
4	296	312			2-3	1120	130	40	3010	−	−	−	−	±
5	236	243	176	176	4‡*	730	50	65	2335	++	−	−	−	++
6	320	308	268	268	7*	1032	94	70	3650	++	++	++	+++	+++
7	234	221	174	174	7-8*	1000	93	48	3095	+	−	−	−	++
8	313	300	267	267	7-8*	973	94	85	4600	+	++	++++	−	+++
9	461	462	334	338	8*	1202	152	42	5611	+++	+++	++++	+++	++++
10	410	380	263	263	9*	1037	108	88	3764	++++	++	+++	++	++++
11	343	370	284	284	11*	1180	130	96	5655	++	+++	++++	+++	++++
12	322	338	235	254	16*	1040	135	80	4980	+	+	++	++	+++
13	363	381	316	352	16*	1206	135	75	5771	±	+++	++++	++	++++
14	354	356	222	222	16*		110	111	4900	++++	++++	++++	++++	+++
15	340	356	277	282	16*	1136	100	63	4800	++	+	++++	++	+++
16	442	444	358	373	16*	1460	160	90	4490	+	±	−	+	+++
						Thyro-parathyroidectomized animals								
17	333	299			2-3	1069	197	103	3753	−	−	−	−	−
18	341	314			2-3	1445	0	69	3391	−	−	−	−	−
19	336	313			2-3	1279	203	63	2733	−	−	−	−	−
20	293	301		272	3-4	1150	90	65	2680	−	−	−	−	−
21	298	297	265	265	4	950	70	50	2725	−	−	−	−	−
22	295	253	220	220	5	1325	68	58	2440	−	−	−	−	±
23	301	301	252	252	6	1180	62	58	2600	−	−	−	−	+
24	295	233	206	206	7-8	833	36	55	2381	−	−	−	−	+
25	409	390	345	345	7-8	1138	47	57	5381	+	−	−	−	+++
26	373	334	254	254	8*	990	45	56	2727	−	−	−	−	+
27	331	319	242	242	9*	1130	45	68	4670	−	−	−	−	+++
28	314	293	212	212	10*	740	0	62	3280	−	−	−	−	++
29	480	440	349	416	16*	1492	164	67	4889	−	−	−	−	+++
30	390	374	297	321	16*	1132	70	36	5650	−	−	−	−	+++
31	341	326	213	213	16*	940	36	40	3290	−	−	−	−	++
32	350	356	293	322	16*	1170	165	42	3960	−	−	−	−	++++
33	314	295	221	259	16*	950	110	40	3360	−	−	−	−	+++
34	301	259	224	275	16*	915	125	36	2280	−	−	−	−	++±
35	381	378	279	325	16*	1050	180	58	4910	−	−	−	−	+++
36	350	328	232	282	16*	1080	60	58	2770	−	−	−	−	+++
37	282	254	215	248	16*	760	74	35	2760	+	−	−	−	+++
38	388	376	272	310	16*	1030	60	50	4670	−	−	−	−	+++

* Killed.
− Normal organ (macro- and microscopically).
± Definite but minor change.
+ Considerable change.
++ Marked change.
+++ Advanced change.
++++ Severest degree of abnormality.

The histological appearance of these lesions was outlined in previous publications (5, 1 and 14).

In addition, every single intact rat with advanced renal injury showed pronounced enlargement of the parathyroid glands (from twice to several times the normal size), whereas the gross and microscopic appearance of the thyroid gland revealed no substantial deviation from the norm. The cytological structure of the parathyroids was found to be consistent with high secretory activity. In rats killed 8–16 days after initiation of the renal injury, sections of the greatly enlarged parathyroid glands (Fig. 2) appeared less intensely stained than normal glands (Hematoxylin-Eosin). This was true of the nucleus as well as of the cytoplasm of the parenchymal cell (Fig. 3). Most nuclei seemed larger than normal and their delicately reticular chromatin structure was replaced by distinct and preponderantly

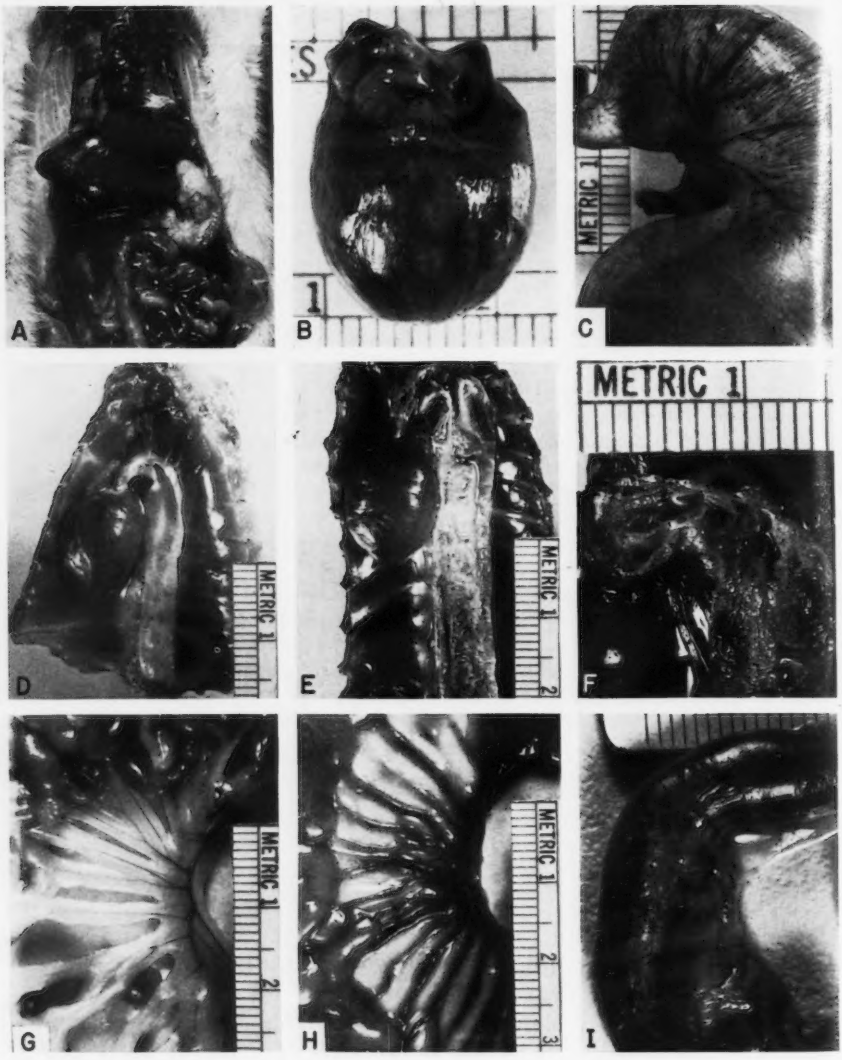

FIG. 1

A. *Thoracic and Abdominal Viscera of Rat*
Survey picture of typical severe organic changes 16 days after renal block. Note the stiff aneurysmic aortic arch, the patchy necrosis on the surface of the left ventricle, the enlarged liver, the enlarged kidney (left of photo), necrosis and calcification of stomach (right of photo) and colon (center).

B. *Close-up View of Rat Heart*
Note patchy necrosis of myocardium (esp. center of picture between the two highlights), six days after renal block.

C. *Close-up View of Rat Stomach*
The necrosis of the muscularis forms a web-like pattern on the surface (same animal as B six days after renal block).

FIG. 2. Cross section in horizontal plane through trachea of adult male rat at height of parathyroid gland 16 days after initiation of standard renal injury. (Animal No. 14 of Table 1.) Note essentially normal picture of thyroid gland and "pale" appearance of large parathyroid gland. (Hematoxylin-Eosin stain, low power.)

D. *Heart and Thoracic Aorta of Rat*
Normal control animal. Note typical thin and elastic aortic wall, diameter 4 mm.

E. *Heart and Thoracic Aorta of Rat*
Treebark appearance of intimal surface of aorta 14 days after renal block, diameter 8 mm.; compare with D.

F. *Aortic Arch Opened, Same as E.*
Note multiple aneurysmic dilatation caused by combination of medionecrosis and hypertension (160 to 180 mm. Hg).

G. *Bloodvessels in Mesentery of Rat*
Normal control animal, compare with H.

H. *Bloodvessels in Mesentery of Rat*
Note strongly enlarged, stiff, calcified bloodvessels 16 days after renal block (same animal as A).

I. *Large Intestine of Rat*
Extensive patchy necrosis of muscularis of ascending colon 7 days after renal block.

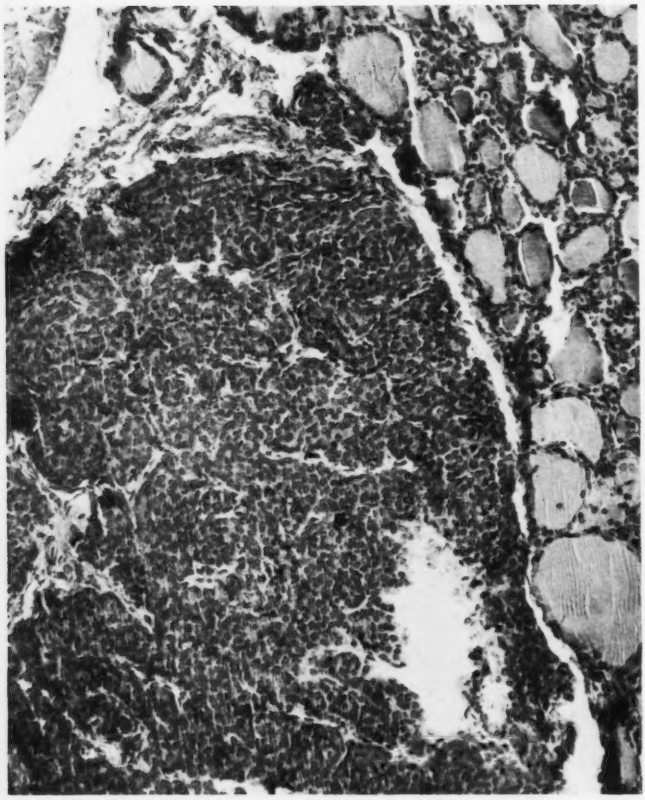

FIG. 3. Same section as Fig. 2. ×60. Note vesicular appearance of nuclei of parathyroid cells and "abundance" of pale cytoplasm.

coarse chromatin particles and a large deeply stained nucleolus (Fig. 4). The rearrangement of the chromatin gave the nucleus a pale, vesicular appearance, an impression which was heightened by the fact that many of these nuclei had lost their normally ovoid structure and had assumed a frankly spherical shape. The cell body as a whole seemed larger, and in some of the cells the abundant homogeneous cytoplasm showed unusually pale staining characteristics. Numerous mitotic figures (nearly one mitosis per high power field) could be readily distinguished in such sections.

In the face of these advanced macroscopic and microscopic changes of the parathyroid gland, quantitative estimation of the enlargement was considered of secondary importance and was deferred in favor of studies promising more direct and conclusive evidence of parathyroid hormone involvement. (Page 287.)

Careful dissection of the operative site and exploration of the entire neck area at autopsy revealed residual parathyroid tissue in animals Nos. 21

23 and 36. Of these, rat No. 21 did not develop renal obstruction and died on the fourth day. It was not included, therefore, in the evaluation. Rat No. 23 showed severe renal damage with minimal tubular calcification, whereas No. 36 despite similar injury to the kidneys had no calcium deposition and behaved like the other completely parathyroidectomized animals with regard to the calcium/phosphate metabolism. It was assumed therefore that these rats had been rendered incapable of producing *excessive* amounts of parathyroid hormone. The presence of some residual parathyroid hormone, possibly in an ectopic accessory gland, was postulated also

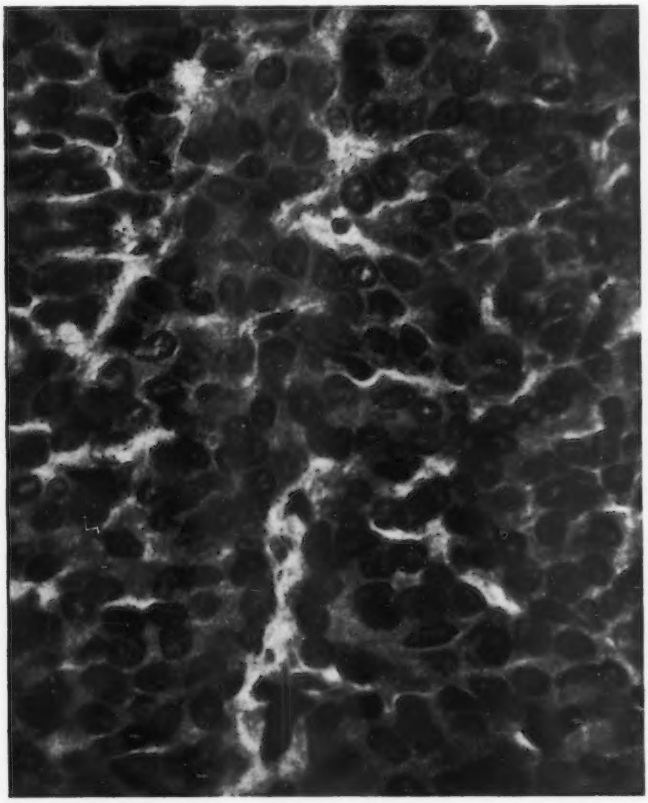

FIG. 4. Same section as Fig. 2. ×450. It can be seen that the vesicular appearance of the nuclei (Fig. 3) is due to rearrangement of the nuclear chromatin into distinct coarse granules. The nuclei vary considerably in size and shape and some of the larger ones have lost their normally ovoid shape and exhibit spherical structure. The few spindle shaped deeply stained structures are nuclei of fibrocytes. Two of these nuclei, located in the approximate center of the photograph, point, like arrows, towards a recently completed nuclear division (left of center) with each of the two young nuclei displaying a centrally located nucleolus.

in rats Nos. 29 and 30 (although no parathyroid tissue could be discovered at autopsy) because both animals exhibited some nephrocalcinosis. (In the overall evaluation, these incompletely parathyroidectomized rats were counted with the TPX animals.)

Calculation of the comparative incidence of muscular lesions in the 24 rats with marked to severe renal damage (Table 2) showed that the complete absence of smooth muscle necrosis in the TPX group contrasted with an incidence of 80% to 90% of mostly severe lesions, especially in the

TABLE 2. PRODUCTION OF MUSCULAR NECROSIS IN THE RAT
Organic changes in 11 intact and 13 thyro-parathyroidectomized (TPX) rats

Organ	Type of Lesion	No. of alterations		Incidence %	
		Intact	TPX	Intact	TPX
Heart	Focal myocardial necrosis and/or diffuse and focal infiltration with mononuclear cells (myocarditis)	11	2*	100	15
Coronary arteries	Medionecrosis and calcification	10	0	91	0
Aorta and large branches	Medionecrosis and calcification	9	0	82	0
Stomach	Necrosis and calcification of muscularis	9	0	82	0
Kidneys	Parenchymatous degeneration, pyelonephritis, interstitial nephritis	11	13†	100	100

* Myocarditis only in one of the two rats.
† No nephrocalcinosis.

arterial tree and the muscularis of the stomach, in intact rats. This difference appears to represent convincing evidence for the protective effect of thyro-parathyroidectomy.

b) *Wet organ weights*

Analysis of wet weights of heart, adrenals, thymus and kidneys of intact and TPX rats and comparison with organ weights of control rats of the same breed and similar body weight (Table 3), brought out the following facts:

Despite the considerable loss of body weight after the renal injury in both intact and TPX rats (about 75 gm. per rat), the wet weights of heart, adrenals and kidneys were substantially above the norm in both experimental groups, with the *intact* rats showing a greater increment in weight than the animals deprived of their thyroid and parathyroid glands. It will be noted that the difference in weight gain between intact and TPX rats was far more pronounced for the adrenals than for heart and kidneys. This is readily seen by comparing the ratio of organ weights (TPX/Intact Rats) in the last column of Table 3.

Statistical analysis revealed that the difference between the weights of these organs of TPX and intact rats was not significant for the kidneys

($P>.10$) and the heart ($P>.30$). With regard to the kidneys this was as expected, since the standard renal injury was equally severe in both experimental groups. Cardiac hypertrophy, in turn, seemed closely linked and directly proportional to the extent of renal damage. The difference of adrenal weights, on the other hand, approaches statistical significance when tested for the total groups ($P<.02$) and is definitely significant when tested for male and female rats separately ($P<.001$).

Similarly the difference in the weight of the atrophic thymus glands in both experimental groups is statistically significant ($P<.001$). On the basis of adrenal cortical hypertrophy alone, one would expect a more marked

TABLE 3. PRODUCTION OF MUSCULAR NECROSIS IN THE RAT
Organic changes as expressed in mean wet organ weights of intact and thyro-parathyroidectomized (TPX) rats

	Normal control		Intact with renal injury		TPX with renal injury		Ratio TPX/intact
No. of rats	9		11		13		
Mean body wt. in gm. Initial	327		355		356		1.0
Mean body wt. in gm. At death	327		280		280		1.0
Mean organ wt.	Mgm.	Mgm./100 gm. body wt.	Mgm.	Mgm./100 gm. body wt. at death	Mgm.	Mgm./100 gm. body wt. at death	
Heart	919	2.81	1126	4.02	1041	3.71	0.87
Thymus	375	1.15	119	0.43	87	0.30	0.73
Adrenals	36	0.11	77	0.28	50	0.17	0.65
Kidneys	2501	7.65	4665	16.66	3990	13.76	0.86

diminution of the thymic weight of the intact group. It should be remembered, however, that maintenance of thymic size is dependent upon the presence of the thyroid hormone (15, and others), so that the significantly greater involution of the thymus in TPX rats, despite the less marked adrenal cortical hypertrophy, could be explained as due to the absence of the thyroid hormone. In addition, recent studies from this laboratory suggest that the size of the thymus gland is greatly influenced by the level of parathyroid hormone activity (16).

Lastly, it should be pointed out that clear-cut separation of thymic tissue from fat and lymph glands is not feasible at autopsy, so that the values of wet weight for this gland are not as accurate as those for the other three organs. This may account in part for the greater fluctuation of thymic weight in individual animals (Table I) and the apparent lack of close correlation between adrenal enlargement and thymic involution.

Histological evaluation indicated that the less marked adrenal enlargement in TPX rats was due to less pronounced cortical hypertrophy. This fits well with the delay in the emergence of hypertension in these animals (to be shown subsequently). It is also in line with our previously reported

finding that muscular necrosis could not be produced in the absence of adrenal cortical hormones (17).

Finally, Table 3 conveys at a glance the striking deviations of the weights of kidneys, adrenals and thymus of experimental animals from those of normal control rats (first column of Table). In each instance the difference is statistically significant ($P<.001$). With regard to the heart, the weight increase is statistically significant for the intact group ($P<.01$) and not significant for TPX rats ($P>.10$). The lesser cardiac enlargement in TPX animals is in line with the less marked adrenal hypertrophy and suppression of hypertension observed in this group.

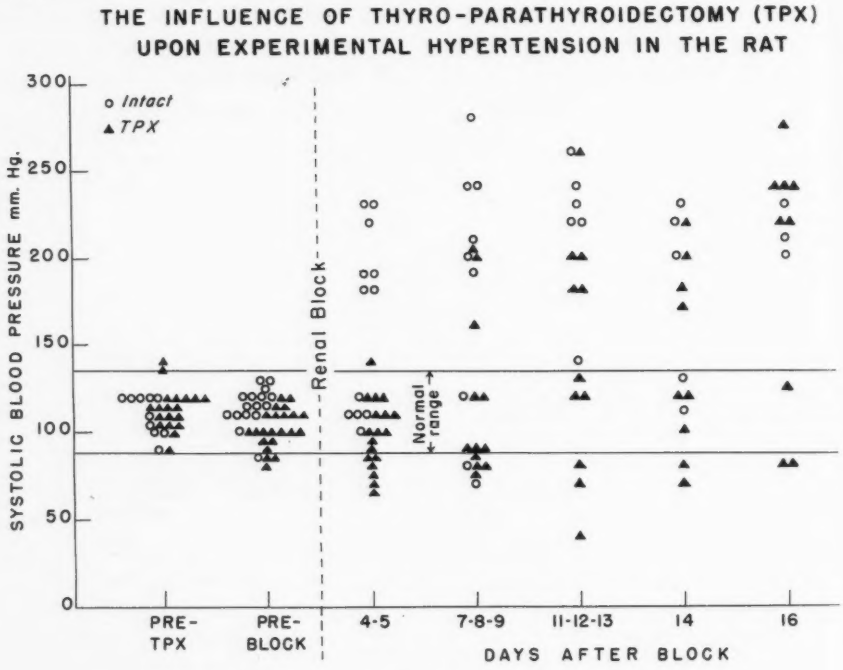

FIG. 5

c) *Systolic blood pressure*

As can be seen from Figure 5, the systolic blood pressure of the normal unanesthetized albino rat was found to lie between 80 and 130 mm. Hg. Removal of the thyroid and parathyroid glands caused a shift towards the lower region of the normal range in about half of the operated animals, as is apparent from the values taken *before* renal block. At the end of the critical period of the first five days following renal injury, when cardiovascular necrosis usually becomes conspicuous, 7 out of 12 intact rats in this particular series were markedly hypertensive, whereas none of 18 TPX

animals showed any elevation above the normal range at this time and 6 of these rats were actually hypotensive. It is only in the second week that some TPX rats reached hypertensive levels.

d) *Changes in the concentration of plasma constituents*

Comparison of data obtained from the plasma of the rats listed in Table 1 revealed conspicuous differences between intact and parathyroidectomized animals primarily with regard to alteration of the calcium and inorganic phosphate levels. This is shown in Table 4, which depicts the mean values of survivors of both experimental groups.

TABLE 4. PRODUCTION OF MUSCULAR NECROSIS IN THE RAT
Changes in plasma constituents of intact and thyro-parathyroidectomized (TPX) rats

Plasma constituents (mean values)	Rat group	Days after renal injury					
		−3	+2	+4 to 5	+7 to 9	+13 to 14	+16
Calcium, mg.%	Intact	9.7	11.6	9.6	10.3	10.4	9.5
	TPX	8.0	7.6	7.7	7.5	8.3	8.3
Inorganic phosphate, mg.%	Intact	4.0	7.5	8.7	13.0	11.5	4.7
	TPX	5.6	8.4	9.3	7.3	5.8	6.2
Total cholesterol, mg.%	Intact	136.0	157.0	199.0	192.0	152.0	139.0
	TPX	145.0	162.0	195.0	193.0	153.0	160.0
Total CO_2 vol., %	Intact	64.1	42.3		58.1	56.9	
	TPX	57.8	47.7		53.0	55.3	
Non-protein nitrogen, mg.%	Intact						88.9
	TPX						70.3

TPX rats had the expected lowered calcium and elevated phosphate level in the plasma *before* the production of the renal injury. Following the kidney block there was initially a further substantial rise in the phosphate concentration and a corresponding further drop in the calcium level. Gradual return towards pre-block values occurred in the course of 2 weeks. Intact rats showed a similar rise of the phosphate concentration in the plasma following the renal obstruction, however, without the concomitant fall of the calcium level. This maintenance of high normal calcium levels was, in fact, the most characteristic difference between intact and TPX animals. It should be stressed that the very high phosphate levels in the intact group on days 7–9 and 13–14 are based on 2 animals only and are therefore not fully representative of the true position of mean values. The remaining survivors had developed "bad" tails, caused by repeated withdrawal of blood and the sample obtained was adequate for calcium determinations only; however, the 16 day values were again based on all survivors (7 intact and 10 TPX rats), since blood was obtained at sacrifice.

It is also apparent from Table 4 that thyroparathyroidectomy per se caused no significant rise in plasma cholesterol levels, whereas renal injury resulted in a marked elevation of the cholesterol concentration in the plasma in TPX as well as in intact rats with levels ranging around 200 mg. % in both groups between the fourth and the ninth day following renal

block. Simultaneously, a transient significant drop occurred in the plasma carbon dioxide content, as a further sign of the serious renal impairment. The plasma non-protein nitrogen levels determined in the survivors of both groups, 16 days after the initiation of renal injury, indicate that kidney function was still abnormal in both groups at that time.

On the whole, the chemical data fully support the gross and microscopic findings that renal damage was about equally severe and of equal duration in both intact and TPX animals.

DISCUSSION

It was demonstrated that in the albino rat the emergence of severe muscular necrosis and calcification in the heart, the gut and especially the arterial tree (mediocalcinosis), following standard renal injury, was completely prevented by prior thyro-parathyroidectomy. It was pointed out that in the evolvement of the characteristic pathologic-anatomical alterations, intact rats manifested marked enlargement of the parathyroid gland with the histological picture of high activity, whereas there was little change in the gross and microscopic appearance of the thyroid gland. On the basis of this observation alone, one is inclined to ascribe to the parathyroid hormone the more important role in the production of muscular damage. To this can be added the well known experimental and clinical evidence (7, 18, 19, and others) that the administration or elaboration of parathyroid hormone in excess may result in a picture closely resembling that of our experimental lesion.

The arterial changes associated with marked hyper-secretion of the parathyroid hormone have been described as consisting of primary necrosis of the media, which under experimental conditions may develop within one to two days after the administration of massive doses of parathyroid extract. It is believed that under the influence of the existing hypercalcemia, rapid and extensive calcification ensues, as calcium is readily precipitated in the alkaline necrotic tissue. A similar process was frequently seen in the myocardium, the mucosa of the stomach and the renal tubules (7). No such damage is known to occur, on the other hand, from excessive *thyroid* hormone administration.

Bergstrand (20) was among the first to demonstrate on the basis of systematic studies, that enlargement of the parathyroid occurs in chronic renal disease and that this enlargement is an expression of hyperfunction. According to Pappenheimer and Wilens (21) most if not all cases of diffuse renal disease are accompanied by significant enlargement of the parathyroid glands. Since this occurs in such varied types of renal disorders as glomerulonephritis, arteriolar nephrosclerosis, hydronephrotic atrophy and suppurative pyelonephritis, they assumed that a common chemical factor stimulates the parathyroid gland to increased activity and growth. These authors as well as Albright and associates (22) and others (23) found a cor-

relation between the gravity of renal disease and the extent of enlargement of the parathyroid glands. In the experimental animal, Pappenheimer (24) demonstrated that restriction of renal tissue in young rats leads to marked increase in the volume of the parathyroid gland. If these partially nephrectomized rats are maintained on a low calcium diet, they develop a picture resembling that of renal rickets in children. Recently Rather (25) reported a high incidence of severe mediocalcinosis of the aorta in adult albino rats with advanced renal disease following renal operations, and suggested secondary hyperparathyroidism as one of the possible causes for this lesion.

The parathyroid hypertrophy in our rats is probably triggered by rising phosphate blood levels following the renal block. The resulting release of excessive amounts of parathyroid hormone, representing the body's effort to hasten renal elimination of phosphate, then causes intoxication of the animal with its own hormone and induces muscular necrosis. In line with this concept, prevention of excessive parathyroid hormone formation should result in absence of tissue necrosis and calcification, as was actually demonstrated in our experiments. It is clear, therefore, why incompletely parathyroidectomized animals, which were apparently still able to produce limited amounts of hormone, seemed to be as well protected as those in which (on the basis of careful gross and histologic examination and the behavior of the calcium and phosphate level in the blood) removal of the parathyroid gland was believed to have been complete.

Evidence for overactivity of the parathyroid glands in our experimental rats with renal injury was seen in the extensive calcification, especially of renal tubules and of the arterial tree, and in the persistence of either high normal or elevated plasma calcium levels in the presence of strongly elevated plasma phosphate concentrations.

In addition, grossly visible enlargement of the parathyroid gland coupled with a histological picture of parenchymatous hypertrophy and hyperplasia as observed under the conditions of our experiments, is in itself strong presumptive evidence of excessive hormone production, since it is believed that under physiological conditions this gland functions only at a fraction of its full capacity. According to Rosof (26) only 5–25% of the parathyroid cells of the rat are in the secretory stage at any one time. Hence three-fourths of the parathyroid tissue can be removed in this species without resulting in apparent lack of secretion. Moreover, the tissue that remains does not undergo a compensatory hypertrophy. Additional evidence, cited in support of the view that the parathyroids operate with a wide margin of safety, is that only extreme mineral imbalance, such as occurs in rickets, will cause a noticeable enlargement of the parathyroids (27).

A protective influence of thyroidectomy could not be excluded in view of the fact that chemical blocking of thyroid hormone formation was effective in diminishing the incidence and severity of muscular necrosis. Reduction

of cellular metabolism and thus of the oxygen requirement of highly active muscle tissue through elimination of thyroid function might conceivably contribute to the prevention of muscular necrosis. Removal of a direct or indirect stimulatory action of thyroid hormone upon other endocrine glands, especially the parathyroid and the adrenal cortex, must also be considered.

It is conceivable that a mechanism of this type is responsible for the delay in the emergence of hypertension in TPX rats since adrenal cortical hormones are of fundamental importance in the production of hypertension, and parathyroid extract in excess is known to raise the arterial blood pressure. Apparently a vasoactive factor can be separated from the renal hemodynamic and calcium mobilizing activities of such preparations (28). The substantial delay in the onset of hypertension in TPX rats may have a bearing upon the prevention of cardiovascular necrosis by obviating the deleterious influence of high intravascular tension at a critical period of intoxication. In intact rats, transient severe hypertension is an almost invariable accompanying sign of such lesions.

A stimulatory effect of the thyroid hormone upon parathyroid activity has been postulated by many investigators. Robertson (29), for example, pointed out that thyroxin reduces the renal threshold for calcium thus leading to increased parathyroid activity, and Engfeldt (30) holds the view that thyroxin, by raising blood phosphorus (directly or indirectly), stimulates the parathyroids to compensatory hyperfunction. Eger (31), on the other hand, in a recent comprehensive review of the relationship of the parathyroids to other glands of internal secretion, makes a point of stressing the unique independence of the parathyroids within the endocrine system. He denies any direct relationship to other endocrine glands except perhaps to the adrenals. Even these ties, according to Eger, are based solely upon parallel activities in the field of mineral metabolism. And yet, it is an undeniable fact that a hyperfunctioning thyroid gland may induce excessive elimination of calcium and phosphorus and that hyperthyroidism may be associated with bone lesions indistinguishable from those occurring in hyperfunction of the parathyroid gland (32).

Our results indicate that while both the parathyroid and thyroid hormone are implicated in the development of experimental muscular necrosis and calcification, autointoxication with parathyroid hormone seems to be the chief responsible factor. It is of particular interest in this connection that recently Crawford and La Zerte (19) in reporting a case of chronic renal disease with secondary hyperparathyroidism make the following statement:

"It was possible to find small foci of necrosis in the myocardium in areas free of detectable calcium (von Kossa stain). This finding suggests strongly that focal myocardial necrosis preceded the calcium depositions and that this sequence explains the unusual severity of the calcification in the myo-

cardium. . . . We can offer no adequate explanation for the focal areas of myocardial necrosis."

A clear-cut delineation of the relative importance of either hormone in the development of experimental muscular necrosis and an elucidation of possible interrelationships of the two hormones or of independent synergistic effects, necessitated additional extensive studies dealing with the *separate* influences of thyroidectomy and parathyroidectomy as well as with various stages of deficiency and excess of the separate and combined hormones. Such studies have been carried out and will be the subject of a subsequent report. Since some of the results have a direct bearing upon the interpretations presented, it seemed justified to anticipate briefly the following clear-cut observations:

In thyro-parathyroidectomized rats substitution with parathyroid extract[2] only, restores the effects of renal injury and induces cardiovascular lesions indistinguishable from those observed in intact rats. Substitution with thyroid hormone only, on the other hand, has no such effect, and the animals remain fully protected against the development of muscular necrosis.

SUMMARY

In the albino rat, the development of severe muscular necrosis and calcification in the arterial tree, the heart and the gut following standard renal injury, can be completely prevented by prior thyro-parathyroidectomy.

Renogenic parathyroid hypertrophy resulting in autointoxication of the intact rat with parathyroid hormone is suggested as the main cause of the muscular injury.

The thyroid hormone is believed to contribute to the development of typical lesions through general elevation of metabolic function or through a more specific stimulatory effect upon other endocrine glands.

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EFFECT OF ESTROGEN ON THYROIDAL IODIDE TRAPPING AND CONVERSION OF INORGANIC I^{131} TO PROTEIN BOUND I^{131} [1,2]

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ESTRUS and the administration of estrogen have been correlated with alterations of thyroid function and structure (1, 2). The mechanism and site of action of ovarian hormones on the thyroid have not been elucidated, although it has often been assumed that thyroidal effects were mediated by the hypophysis and thyrotrophin (3, 4, 5, 6, 7 and 8). This investigation was undertaken to define more precisely the locus of action of estrogen on thyroid function. For this purpose the effect of estrogen on the "iodide trapping" mechanism of the thyroid and on the conversion of inorganic I^{131} to protein bound I^{131} was studied in castrate male and hypophysectomized female rats.

MATERIALS AND METHODS

Male rats (Holtzman) were castrated, and female rats were hypophysectomized (Hormone Assay Laboratories, Chicago, Illinois) 10 to 20 days before use. The control and experimental groups of animals for each experiment weighed approximately the same at the start of an experiment and were studied simultaneously. The experimental rats were given 500 I. U. of estrogen[3] in 0.2 ml. of saline daily subcutaneously, for 3 days. Controls received 0.2 ml. of saline daily subcutaneously for the same period of time.

I^{131}, obtained from the Oak Ridge National Laboratory, Oak Ridge, Tennessee, was administered intraperitoneally on the day of the last estrogen or saline injection either $1\frac{1}{2}$, 24, or 48 hours before sacrifice. For the study of thyroidal:serum I^{131} concentration ratio (T/S), 2 mg. of tapazole[4] were injected subcutaneously one hour before the administration of the isotope.

The animals were etherized and exsanguinated. Their thyroids were dissected out, cleaned of adventitial tissue, and weighed to the nearest 0.1 mg. on a Roller-Smith torsion balance. The glands were counted in a well-type thallium activated sodium iodide crystal counter having a sensitivity of 1×10^6 counts per minute per microcurie of I^{131}. The thyroids were then homogenized by mortar and pestle in 0.2 ml. of distilled water and washed twice with 0.4 ml. of distilled water each time. The blood collected from the heart at autopsy was allowed to clot and 1 or 2 ml. of serum was removed for counting. Balanced $ZnSO_4$ and $Ba(OH)_2$ solutions were used to precipitate the proteins of the thyroid

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[3] Theelin, aqueous, Parke, Davis & Co. 1 ml. contains 5 mg. or 50,000 I.U. of estrogen.

[4] Tapazole (1-Methyl-2-Mercaptoimidazole) was generously supplied by Eli Lilly & Co., Indianapolis, Indiana.

homogenates and sera, as described by Somogyi (9). The precipitates were alternately centrifuged and washed four times in 0.1% KI solution. This method was found to eliminate 96 to 98% of I^{131} added to sera or thyroid homogenates. After washing, the thyroid and sera precipitates were counted. All counts were corrected for decay and background and were at such a level that the sampling error was less than 2%. Two standard solutions of I^{131}, containing the same number of counts as was injected into the rats, were appropriately diluted and counted in duplicate for each experiment.

RESULTS

A. *Effect of Estrogen on "Iodide Trapping"*

"Iodide trapping" was determined by calculating T/S ratios in animals 1½ hours after the administration of I^{131} intraperitoneally, as suggested by

TABLE 1. EFFECT OF ESTROGEN ON THYROIDAL UPTAKE OF I^{131} AND THYROIDAL:SERUM I^{131} CONCENTRATION RATIO (T/S)

	No. of rats	Body weight, gm.	Thyroid weight, mg.	Uptake per 100 mg. thyroid, % of dose	Serum I^{131}, c/m/0.1 ml.	T/S
Castrate						
Estrogen	7	210	9.8±0.43	*2.25±0.12*	1810±74	*28.0±2.14*
Control	7	235	8.7±0.24	1.10±0.12	1613±94	18.1±1.61
Hypophysectomized						
Estrogen	7	128	7.1	*1.6 ±0.16*	2051±74	*19.3±2.24*
Control	7	140	7.8	0.34±0.05	1900±46	4.4±0.6

± Figures represent standard error of mean.
Italicized figures are significantly different from controls at 1% level.

Vanderlaan and Greer (10). Tapazole, given one hour prior to I^{131}, effectively prevented the conversion of inorganic I^{131} to protein bound I^{131} (PBI^{131}).

In Table 1 the results of these experiments are presented. The T/S ratios were 1.5 and 4 times higher in estrogen treated castrate males and hypophysectomized females, respectively, than the T/S ratios of control rats. Thyroidal uptake of I^{131} was also 2 to 4 times greater in estrogen treated animals than in controls, for castrate and hypohysectomized rats, respectively. Serum I^{131} concentration (counts per minute per 0.1 ml. of serum) was approximately 10% higher in the experimental groups than in controls, perhaps due in part to the smaller size of the estrogen treated animals. The protein bound I^{131} in the thyroids and sera of these animals was counted but was found to be negligible. The data were not recorded here.

B. *Effect of Estrogen on Conversion of Inorganic I^{131} to Protein Bound I^{131}*

The data of these experiments are recorded in Table 2. The salient observations were as follows:

a) In all instances, thyroidal uptake of I^{131} was significantly greater in estrogen treated rats than in controls.

b) Eighty to ninety-four percent of the inorganic I^{131} was converted in the thyroids of all rats to PBI^{131}. In castrate males the ratio of thyroidal

PBI^{131} to total thyroidal I^{131} ($\times 100$) was the same at $1\frac{1}{2}$ hours as at 24 and 48 hours after administration of the isotope. In hypophysectomized animals the ratio of thyroidal PBI^{131} to total thyroidal I^{131} ($\times 100$) was slightly less than $1\frac{1}{2}$ hours than at 24 and 48 hours.

c) The proportion of total thyroidal I^{131} converted to PBI^{131} was the same in estrogen treated and control groups.

TABLE 2. EFFECT OF ESTROGEN ON CONVERSION OF I^{131} TO PROTEIN BOUND I^{131} IN THYROID AND SERUM

	No. of rats	Body weight, gm.	Thyroid weight, mg.	Uptake per 100 mg. of thyroid, % of dose	Thyroid PBI^{131} / Thyroid I^{131} $\times 100$	Serum PBI^{131} c/m/ml.
1½ Hours After Injection						
Castrate						
Estrogen	7	299	11.9	*27.9 ± 1.55*	91.8	136
Control	7	317	11.3	12.8 ± 1.18	91.2	138
Hypophysectomized						
Estrogen	6	145	5.9	*19.1 ± 2.82*	81.2	376
Control	4	156	5.5	8.1 ± 1.86	79.3	320
24 Hours After Injection						
Castrate						
Estrogen	7	224	8.4	*160.0 ± 19.0*	92.6	*1249 ± 131.0*
Control	7	265	8.1	71.0 ± 10.2	92.4	786 ± 70.0
Hypophysectomized						
Estrogen	9	140	7.6	*20.5 ± 2.0*	89.6	1022 ± 37.5
Control	11	142	8.0	6.5 ± 0.56	94.1	974 ± 36.6
48 Hours After Injection						
Castrate						
Estrogen	7	187	9.1	*104.0 ± 5.66*	92.1	*2751 ± 318*
Control	7	219	9.1	50.0 ± 2.88	90.2	1621 ± 145
Hypophysectomized						
Estrogen	7	143	7.5	*16.2 ± 2.30*	88.9	1776 ± 124.8
Control	7	146	7.5	7.7 ± 0.73	88.1	1536 ± 94.4

± figures represent standard error of mean.
Italicized figures are significantly different from controls at 1% level.

d) At 24 and 48 hours after I^{131} administration, serum PBI^{131}, expressed as counts per minute per ml. of serum, was greater in estrogen treated castrate and hypophysectomized animals. At $1\frac{1}{2}$ hours the serum PBI^{131} counts were negligible and subject to inaccuracy of counting.

DISCUSSION

The present experiments have clearly demonstrated that estrogen affected iodine metabolism, presumably by direct action upon the thyroid and without the mediation of the pituitary.

The "iodide trapping" was enhanced by estrogen. Under the influence of the ovarian hormone thyroids were able to extract one and one-half to four times more inorganic I^{131} from the blood than control thyroids. In part, the enhanced "iodide trapping" elicited by estrogen may be explained by the higher serum concentration in estrogen treated rats. However, an increase of 10% in the serum I^{131} concentration could not account by itself for a two to fourfold increase of T/S ratios and of thyroidal I^{131} over control values.

Estrogen increased the thyroidal radioiodine content, both inorganic and organic. A comparison of thyroid weights between estrogen treated and control animals revealed no significant differences. The quantity of I^{131}, inorganic and organic, was more than doubled in the hormone treated groups. Consequently, the total I^{131} and its concentration were increased within the thyroid by the injection of estrogen.

The absolute quantity (concentration) of PBI^{131} appearing in the serum 24 or 48 hours after administration of I^{131} (expressed as counts per minute per ml.) was greater in the estrogen treated animals than in controls. This might have been accomplished by a decrease of plasma volume but evidence to date has indicated that the ovarian hormone enlarges the plasma space (11). An increased serum PBI^{131} might also have been achieved by diminished peripheral utilization of serum PBI^{131}. There have been no data concerning PBI^{131} utilization under the influence of estrogen. Engstrom and Markardt (12) have reported that the administration of estrogen elevated serum protein bound iodine in human beings.

The action of estrogen on the thyroid was obtained without the mediation of the pituitary and it was assumed that the ovarian hormone acted directly upon the thyroid. The hypothesis has often been proposed that the effects of estrogen and estrus on thyroid physiology were achieved via the pituitary, by increasing production and discharge of TSH (3–8). Such an hypothesis remains to be proved. Nothing in this paper favors or disproves this assumption, but the data reveal that estrogen exerted an action on thyroid function in the absence of the pituitary.

SUMMARY

The administration of estrogen to castrate male and hypophysectomized female rats significantly enhanced "iodide trapping" in the thyroid. In addition estrogen increased the thyroidal I^{131} and the quantity of thyroidal PBI^{131}. The concentration of serum PBI^{131} was absolutely greater in estrogen treated rats than in controls.

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THE EFFECT OF GRADED HYPOPHYSECTOMY ON THYROID, GONADAL, AND ADRENOCORTICAL FUNCTION IN THE DOG[1]

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IT IS well known that the removal of three quarters of the anterior lobe of the pituitary often results in no detectable endocrine deficiency, and that in order for the usual atrophic endocrine changes that follow hypophysectomy to appear, removal of the pituitary must be essentially complete. On the other hand, relatively little attention has been paid to the effect on the target endocrine organs of removal of more than 75% but less than all of the anterior pituitary. Because of growing interest in the effect of anterior pituitary transplantation and hypothalamic lesions on endocrine function, this problem is of considerable interest and importance. The present study was therefore undertaken to determine the effect of removing graded amounts of anterior pituitary tissue on gonadal, thyroid and adrenocortical structure and function in the dog.

MATERIALS AND METHODS

Thirty adult mongrel dogs were used in this study. They were maintained on a diet of *Ken-L-Biskit*.[4] All but five of the animals were males. Hypophysectomies were performed by the transbuccal or the transtemporal approach and the animals allowed to recover. Nineteen dogs were killed and autopsied six weeks after hypophysectomy, three dogs nine weeks postoperatively, and the remainder, 4–6 months after operation. In all dogs, the gonads, thyroids and adrenals were examined histologically. In 25 animals, these organs were also weighed and their weights compared to values seen in the normal dog. In addition to these anatomical studies, the thyroidal uptake of radioactive iodine was determined in 24 dogs, the hypertrophic response of the remaining adrenal to unilateral adrenalectomy was observed in 17, the effect of immobilization stress on plasma 17-hydroxycorticoid observed in nine, and the adrenal venous corticoid output determined in six dogs.

Radioactive iodine uptakes were determined by a technic described in detail elsewhere (1). The change in adrenal weight induced by unilateral adrenalectomy was tested by removing the left adrenal gland four weeks after hypophysectomy, permitting the dog to recover, and determining the right adrenal weight two weeks later. In the

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normal dog, this procedure results in a 20% increase in the size of the remaining gland (2). The response to psychic stress was observed by immobilizing the dogs in a canvas sling stand for two hours. Bloods were drawn from the femoral artery before the procedure and at the end of immobilization for 17-hydroxycorticoid determination. In the normal dog, this procedure results in a mean increase in plasma 17-hydroxycorticoids of 22 μg.% (3). Right adrenal cannulations were performed by the technic of Hume and Nelson (4). Adrenal venous blood samples were collected for 4–6 minutes. As a control for this procedure, six normal dogs which had been subjected to left adrenalectomy two weeks previously had their right adrenal veins cannulated, and samples collected.

All blood samples were centrifuged and the plasma stored in the frozen state until corticoid determinations were done. 17-hydroxycorticoids were estimated by the technic of Silber and Porter (5), and expressed as μg.% of plasma in the case of peripheral samples. Adrenal venous corticoid output was calculated by multiplying the observed corticoid concentration per ml. of plasma by the minute blood flow and dividing by two, assuming a hematocrit of 50%.

At autopsy, all animals were perfused through the carotid arteries with saline followed by 10% neutral formalin. The pituitary-hypothalamic area was removed in one piece, being careful to include in the pituitary area the dura overlying the sphenoid bone and all tissue between it and the hypothalamus. The blocks of tissue from the hypothalamic area were then sectioned serially at 20 micra and stained with Bullard's modification of the hematoxylin and eosin stain. The thyroids and gonads were sectioned at 10 micra and stained with hematoxylin and eosin. Adrenals were stained with hematoxylin and eosin. Frozen adrenal sections were also stained by the technics described by Greep and Deane (6). Tracings were made of every third section of the pituitary-hypothalamic area, using a projector which magnified nine times. The tracings were then compared to similar tracings of a normal dog pituitary and the percentage of anterior lobe tissue remaining estimated visually by this means.

RESULTS

In Figure 1, the dogs have been classified by the presence or absence of atrophy of the target endocrine organs studied. Thyroid atrophy was judged to be present when a markedly flattened epithelium was seen and no reabsorption lacunae were visible in the colloid. Ovaries were judged to be atrophic when only immature follicles were present and active corpora lutea were absent. Testes were judged atrophic when spermatogenesis did not proceed to the formation of adult sperm in any of the tubules examined, and when definite atrophy of the Leydig cells was present. In addition, testicular atrophy was arbitrarily judged to be present when the combined weight of both testes was less than one gram per kg. of body weight. The presence of adrenocortical atrophy was determined by observing thinning of the cortex, involution in the cells of the fasciculata and reticularis, and loss of the normal columnation of cells along the sinuses of the fasciculata. In addition, individual adrenal glands were judged to be atrophic when they weighed less than 40 mg./kg. of body weight. Because of the considerable overlap in ovarian and thyroid weight in completely hypophysectomized and normal dogs, weight was not used as a criterion of the presence or absence of atrophy in the case of these organs.

Using these criteria, six animals failed to show any gonadal, thyroid or adrenocortical atrophy, while 19 animals showed definite atrophy in all

three of these endocrine organs. Three animals showed definite gonadal atrophy without thyroid or adrenal change, and two animals who had gonadal and thyroid atrophy had normal adrenal cortices.

The 24 dogs in whom thyroid function was evaluated by determining the uptake of radioactive iodine are presented in Figure 2. They were arbitrarily divided into those with uptakes greater than 8% of the injected dose at 72 hours and those with uptakes of less than 8%. It will be noted that none of the animals with uptakes in the normal range (greater than 8%) had adrenal atrophy. On the other hand, 16 of the 20 dogs with depressed

EFFECT OF GRADED HYPOPHYSECTOMY ON THE HISTOLOGY AND WEIGHT OF THYROIDS, GONADS, AND ADRENALS IN THE DOG

No. Dogs	Gonadal Atrophy	Thyroid Atrophy	Adrenal Atrophy
6	0	0	0
3	+	0	0
2	+	+	0
19	+	+	+

FIG. 1. 0: indicates atrophy absent, +: atrophy present.

I^{131} UPTAKE < 8% AT 72 HOURS

NO. DOGS	GONADS ATROPHIC	ADRENALS ATROPHIC
1	0	0
3	+	0
16	+	+

I^{131} UPTAKE > 8% AT 72 HOURS

NO. DOGS	GONADS ATROPHIC	ADRENALS ATROPHIC
2	0	0
2	+	0

CORRELATION BETWEEN DEPRESSED I^{131} UPTAKE AND GONADAL AND ADRENAL ATROPHY IN DOGS AFTER GRADED HYPOPHYSECTOMY

FIG. 2. 0: indicates atrophy absent, +: atrophy present. Thyroidal radioactive iodine uptakes (I^{131} uptakes) are expressed as % of the injected dose of I^{131} present in the thyroid gland at 72 hours.

iodine uptakes had adrenal and gonadal atrophy and 3 had only gonadal atrophy. The one animal which failed to show gonadal changes in the presence of depressed thyroid function is of interest because the histology of this animal's thyroid gland was normal, while his radioactive iodine uptake was 5.4%. In a previously published series of normal dogs, one animal with an uptake of 5% at 72 hours was observed although the mean uptake for the group was 32% (1). Probably, therefore, thyroid function was actually normal in this animal. In all instances except this one, a radioactive iodine uptake of less than 8% was associated with marked flattening of the thyroid epithelium, while uptakes of over 8% were associated with a normal histologic appearance of the thyroid gland.

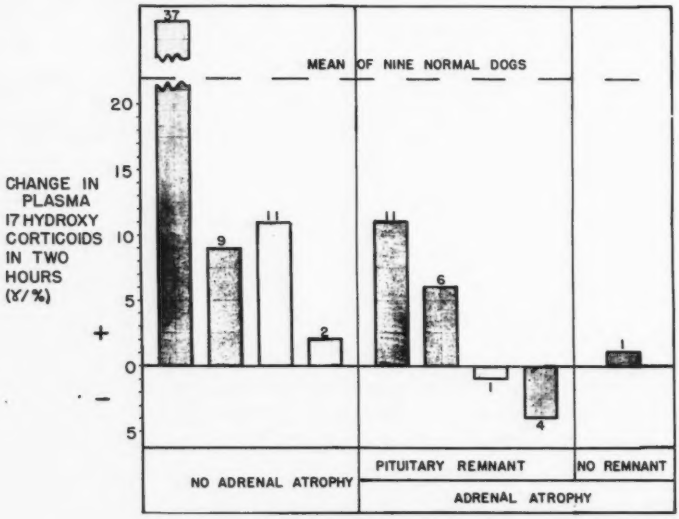
The results of the studies aimed at evaluating adrenocortical function

CHANGES IN SIZE OF THE REMAINING ADRENAL AFTER UNILATERAL ADRENALECTOMY IN THE PARTIALLY HYPOPHYSECTOMIZED DOG

EFFECT OF UNILATERAL ADRENALECTOMY ON THE REMAINING ADRENAL	No. DOGS	% WITH PITUITARY REMNANTS	MEAN LEFT ADRENAL WEIGHT (mg./kg.)
> 8% WEIGHT INCREASE	9	100%	31.1
< 8% WEIGHT INCREASE	4	0%	31.0
> 8% WEIGHT INCREASE	4	100%	50.4
< 8% WEIGHT INCREASE WITHOUT INITIAL ATROPHY	0	—	—

FIG. 3. Dogs were subjected to left adrenalectomy two weeks before removal of the right adrenal. Percent change in weight was calculated by subtracting the weight of the left adrenal, in mg./kg. from the weight of the right, and dividing this value by the weight of the left adrenal, expressing the answer as a percent.

are summarized in Figures 3, 4 and 5. The unilaterally adrenalectomized animals have been arbitrarily divided into those with a greater than 8% increase in the weight of the remaining adrenal and those in whom the response was less than 8%, because in previous experiences with normal dogs,



PERIPHERAL PLASMA CORTICOID RESPONSE TO IMMOBILIZATION STRESS IN PARTIALLY HYPOPHYSECTOMIZED DOGS

FIG. 4. For definition of "adrenal atrophy," see text.

an 8% increase in adrenal weight is the lower limit of the response observed (2). By these criteria, four dogs failed to show a significant response. On the other hand, 13 animals showed definite increases in adrenal weight. In four of this latter group of dogs, the left adrenal gland which was removed two weeks before the right failed to show any significant atrophy. The remaining nine animals showed definite atrophy of the removed left adrenal, the extent of the atrophy being comparable to that observed in the animals which failed to respond to this stress. Interestingly, in all cases, the animals which showed hypertrophy of the remaining adrenal after unilateral adrenalectomy had definite pituitary remnants present, while the four animals

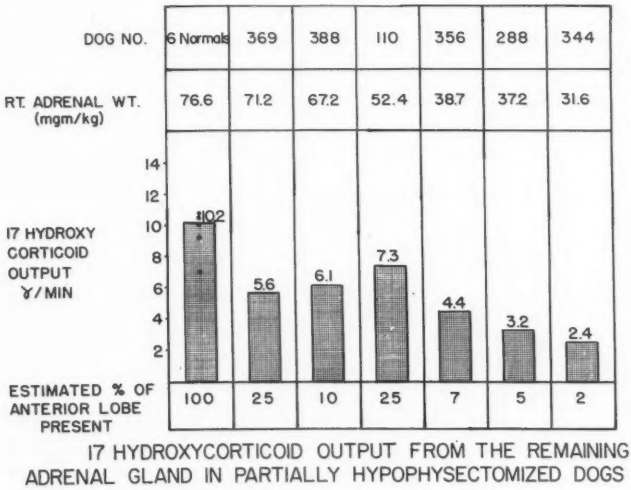


FIG. 5. In the first column, the individual dots represent individual values, while the height of the bar and the figure on top of the bar indicate the mean. In the remaining columns, the height of the bar indicates the actual output in the individual dogs identified by the numbers in top line.

which failed to show the response were in all cases completely hypophysectomized. It, therefore, seems clear that small pituitary remnants are capable of stimulating the adrenal even in the presence of extensive adrenocortical atrophy (Fig. 3).

The change in plasma 17-hydroxycorticoids after two hours of immobilization stress also indicated a capacity of the atrophic adrenal to respond, at least partially, to increase ACTH secretion. Four animals without adrenocortical atrophy showed increases of 17-hydroxycorticoid secretion ranging from 2–37 μg.%. Two animals with adrenocortical atrophy showed increases of 6 and 11 μg.% while two other animals had significant corticoid material present in the control samples, but were unable to increase this level with stress. All had pituitary remnants present. In the one animal

tested which had adrenal atrophy and no pituitary remnant, the control level was zero and the two hour value one gamma percent (Fig. 4).

The 17-hydroxycorticoid output per minute from the remaining right adrenal gland in six of these animals is presented in Figure 5. It will be noted that there is a rough correlation between the magnitude of the hormone output and the degree of adrenocortical atrophy present. On the other hand, however, in the three dogs which showed unequivocal adrenocortical atrophy, 17-hydroxycorticoid outputs of 4.4, 3.2, and 2.4 μg./min. were observed. All these animals had small but definite remnants of anterior pituitary tissue present. This series of animals does not include any in whom the hypophysectomy was complete. However, data have been ob-

TABLE 1. CORRELATION BETWEEN ENDOCRINE ABNORMALITIES SEEN IN PARTIALLY HYPOPHYSECTOMIZED DOGS AND THE SIZE OF THE ANTERIOR PITUITARY REMNANT. THE PER CENT OF ANTERIOR LOBE REMAINING WAS VISUALLY ESTIMATED BY COMPARING TRACINGS OF THE SERIAL SECTIONS OF THE PITUITARY-HYPOTHALAMIC AREA IN EACH DOG TO SIMILAR SECTIONS OF A NORMAL ANIMAL OF THE SAME SIZE

Endocrine pattern	Estimated per cent of anterior lobe remaining		Number of dogs
	Mean	Range	
No endocrine abnormality	27	10–40	6
Atrophic gonads	11	3–25	3
Atrophic gonads and thyroid	5	5	2
Atrophic gonads, thyroid, and adrenals			
a. response to unilateral adrenalectomy present	3	1–7	9
b. response to unilateral adrenalectomy absent	0	0	4
c. response to unilateral adrenalectomy not tested	2	0–4	6

tained on 15 completely hypophysectomized dogs as part of other studies, and in these 15 animals, 17-hydroxycorticoid output by this particular technic has been less than one μg./min. in all instances and zero in all but three of the dogs. Extensive experience with this technic of cannulation in hypophysectomized dogs by Hume and Nelson (4), but using a different 17-hydroxycorticoid method, also indicates that a complete hypophysectomy is associated with a 17-hydroxycorticoid output of less than one μg./min.

The correlation between the amount of remaining pituitary tissue and the presence or absence of changes in the target endocrine organs was determined by dividing the animals into the four groups listed in Table 1 and determining for each the mean percentage of anterior lobe tissue remaining, and the percentage range. In addition, the animals with thyroid, gonadal and adrenocortical atrophy have been subdivided into those with and those without a hypertrophic response in the remaining adrenal after unilateral adrenalectomy. The estimated mean amount of remaining anterior lobe was 27% in the animals who failed to show endocrine abnormality, the values ranging from 10–40%. In the animals with gonadal depression alone, the mean was 11 with a range of 3–25%. Both the animals

with gonadal and thyroidal insufficiency without adrenocortical insufficiency had 5% of their anterior lobes remaining. The animals with atrophic adrenals who showed a response to unilateral adrenalectomy had 1–7% of their anterior pituitary present, the mean value being 3%. As previously noted, in animals who failed to respond to unilateral adrenalectomy, the hypophysectomy was invariably complete. In the remaining six animals who were not subjected to unilateral adrenalectomy but who showed atrophy of all three target endocrine glands, the percentage of pituitary tissue remaining ranged from 0–4% with a mean of 2%. There is, thus, a reasonably good correlation between the amount of pituitary tissue left in situ and the observed effects, although in a number of instances there is some overlap between the groups. The pituitaries of these animals were not examined in detail with special staining technics. In general, however, hematoxylin and eosin stain showed that both eosinophils and basophils were present in the pituitary remnants of the animals with partial endocrine deficiencies.

DISCUSSION

Smith, in a classic paper published in 1932, (16) showed that removal of 70% of the anterior pituitary from the rat was associated with no consistent change in the weight or histology of the thyroids, gonads, or adrenals. On the other hand, when 90% of the gland was removed, gonadal deficiency was uniformly present while thyroid and adrenocortical histology was usually intact. Keller and his associates have studied this problem in the dog and have also concluded that with increasingly complete pituitary removal, gonadal function is first to suffer while adrenocortical is last (7, 8, 9). In a number of dogs, these observers found that when the hypophysectomy included damage to the hypothalamus, adrenocortical atrophy was not present, whereas it was when the hypophysectomy was complete but did not damage the hypothalamus. They also admitted, however, that in all the animals in whom this lack of atrophy was observed, small pituitary remnants were found in the scar tissue underlying the hypothalamus. In view of the present studies, these remnants probably explain the absence of adrenal changes.

There are obviously not enough dogs in the group with selective endocrine insufficiency in the present series to permit definite conclusions. On the other hand, it is probably significant that in this series of 30 dogs, no instance of isolated adrenocortical or thyroidal depression has been seen, and the sequence of loss of endocrine function has been gonad-thyroid-adrenals. It should be emphasized further that there is undoubtedly considerable overlapping in the loss of these functions. For instance, the gonadal atrophy seen in the animals with gonadal deficiency alone was in no instance as marked as that observed in the animals who also had thyroidal and adrenal involvement. Similarly, because of the criteria used in

this study, a mild decrease in adrenocortical weight without clearcut atrophic changes could occur, indicating some depression of ACTH secretion, and still remain within the classification of normal adrenocortical function.

The reason that one endocrine organ should show evidence of deficiency before another is unknown. It is undoubtedly partly due to the choice of indices used in evaluating that particular gland. In addition to this, however, it is possible that there is a difference in end organ sensitivity to the particular trophic hormones involved. It is also possible that there is a difference in the amount of secretion of the various trophic hormones from the pituitary, or even that production of particular trophic hormones is localized in various parts of the anterior pituitary, the cells secreting ACTH being in or near the pars tuberalis. In general, however, there is little histologic evidence to support this latter idea. At present, all that can be said is that more pituitary tissue is needed to support gonadal function than for thyroids and that adrenocortical maintenance requires least of all.

In man, progressive pituitary disease is usually associated first with symptoms of gonadal depression, and then with evidence of thyroid and adrenocortical atrophy (10, 11). On the other hand, there are many well documented cases in the literature which seem to indicate selective failure of the pituitary to secrete thyrotropin or ACTH (12, 13). These cases are not necessarily in disagreement with the animal data, because the pathologic situation, particularly in humans with pituitary tumors is quite different from that obtaining in the animal subjected to surgery. Thus, in the human, it is usually impossible to evaluate the effects of pressure from the tumor on the surrounding gland, and involvement of the infundibulum or even the hypothalamus cannot be ruled out except at autopsy.

The finding that the atrophic adrenal is still capable of a considerable degree of function if a small pituitary remnant is present is of considerable interest. Destruction of the median eminence of the hypothalamus in the dog is associated with an inability of the adrenal gland to secrete 17-hydroxycorticoids in response to stress but does not produce adrenocortical atrophy (14). The present study therefore constitutes evidence that the observed effects of hypothalamic lesions on the adrenal cortex are not due to pituitary damage per se. Furthermore, unlike the animals in this series, selective thyroidal or adrenocortical depression and the occurrence of thyroid and adrenocortical abnormalities with normal gonadal weight and histology has been repeatedly observed in animals with hypothalamic lesions (15).

SUMMARY

The effect of partial removal of the anterior pituitary on gonadal, thyroidal, and adrenocortical structure and function has been observed in 30 dogs. Removal of about three-quarters of the anterior pituitary results in no detectable endocrine abnormality. Removal of 97–99% of the pitui-

tary is associated with atrophy of all three target glands. Removal of 75–95% of the anterior lobe is associated with gonadal or gonadal and thyroidal insufficiency while isolated thyroidal and adrenocortical insufficiencies have not been observed in this series.

In animals with small pituitary remnants, the adrenal is capable of at least a moderate degree of function in spite of extensive atrophy, as indicated by an increase in weight of the remaining adrenal after unilateral adrenalectomy, an ability to increase peripheral blood 17-hydroxycorticoid levels in response to immobilization stress, and an output of 17-hydroxycorticoids from the adrenal vein at an appreciably higher level than that seen following total hypophysectomy.

Acknowledgments

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THE EFFECT OF UNILATERAL ADRENALECTOMY ON ADRENAL VENOUS 17-HYDROXYCORTICOSTEROID OUTPUT IN THE DOG[1]

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IT HAS been assumed that the hypertrophied adrenal cortex is capable of a greater steroid output than the normal, but direct proof of this assumption has been lacking. Recently, Hechter, Macchi, Korman, Frank and Frank (1) reported extremely wide variations in 17-hydroxycorticosteroid output from the adrenals of a series of acutely stressed dogs without apparent correlation to the size of the glands. In view of this variation, it seemed of interest to determine whether or not the hypertrophy of the remaining adrenal cortex induced by unilateral adrenalectomy was associated with a higher output of cortical hormones than that seen in the normal animal under identical conditions of surgical stress. Therefore, the output of 17-hydroxycorticoids in the adrenal venous blood from the remaining adrenal one week after unilateral adrenal vein cannulation and adrenalectomy has been studied, and compared to the cortical hormone output in a similar group of dogs which one week previously had been subjected to unilateral adrenal vein cannulation without adrenalectomy. The hormone output of the remaining adrenal gland two hours after unilateral adrenalectomy has also been studied.

MATERIALS AND METHODS

Eighteen 10–15 kg. adult mongrel dogs of both sexes were used in this study. They were maintained on a diet of Ken-L-Biskit.[4] Adrenal vein cannulations under ether anesthesia were performed under sterile conditions according to the technique of Hume and Nelson (2). This permitted intermittent collection of the total venous outflow from the adrenal, while between samples, adrenal venous blood flowed into the vena cava in a normal fashion.

The dogs were divided into three groups of six animals each. In the animals in group one, the left adrenal vein was cannulated, samples obtained, the gland removed, and the wound sutured. One week later, the right adrenal vein was cannulated and the right

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adrenal was removed. The animals in group two were similarly subjected to left adrenal cannulation, but the wound was sutured without removing the adrenal gland, and one week later, the right adrenal vein was cannulated and the gland removed. Left adrenal vein cannulation and left adrenalectomy was performed in the animals in group three. Their wounds were then sutured, and immediately thereafter the right adrenal vein was exposed and cannulated. Right adrenal venous samples were then collected exactly two hours after removal of the left adrenal, and the right adrenal was then removed.

In all instances, five minute samples of adrenal venous blood were obtained, and the animals were then given one international unit of ACTH in two ml. of saline intravenously. Three minutes later, another five minute adrenal venous sample was obtained in all the dogs.

All adrenal venous blood samples were collected in heparinized tubes. They were centrifuged immediately and the plasma stored in the frozen state. 17-hydroxycorticoid determinations were performed by the method of Silber and Porter (3). Corticosteroid output was calculated by multiplying the corticoid content per ml. of plasma by the adrenal blood flow per minute for each sample, and dividing the result by two, assuming a hematocrit of 50%.

The removed adrenal glands were dissected clean immediately, washed with saline and weighed on a laboratory balance. The glands were then fixed in formalin and sectioned for histologic study.

RESULTS

In the animals subjected to left adrenal cannulation and left adrenalectomy one week before right adrenal cannulation (Group one), the mean left adrenal corticosteroid output was 7.2 ± 0.8 μg./min. (standard error of the mean). Mean right adrenal output in these animals was 10.2 ± 0.8 μg./min. One of these animals failed to show a greater output from the right adrenal than from the left, but in the remaining five dogs, the output on the right side was 2.7 to 5.8 μg./min. greater than the left. On the other hand, the animals subjected to right adrenal vein cannulation without left adrenalectomy (Group two) had a mean left adrenal corticosteroid output of 7.2 ± 0.6 μg./min. and a mean right adrenal output one week later of 6.4 ± 0.5 μg./min. When left adrenal vein cannulation and left adrenalectomy was followed in two hours by cannulation of the right adrenal vein (group three), the mean left adrenal corticosteroid output was 5.9 ± 0.8 μg./min., and the mean right adrenal output 4.6 ± 0.8 μg./min. (Table 1). The difference between mean right adrenal output in group one and that in groups two and three is highly significant ($P < 0.01$), while the difference between Group two and Group three is not statistically significant, nor is the variation in mean left adrenal output between any of the groups.

The mean right adrenal weight in the dogs subjected to unilateral adrenalectomy one week before the right adrenal was weighed was 76.6 ± 0.8 mg./kg. In the animals in which the left adrenal vein was cannulated but the adrenal not removed, the mean right adrenal weight was 62.9 ± 1.0 mg./kg., and the mean weight of the right adrenals removed two hours after left adrenalectomy was 55.9 ± 1.0 mg./kg. Histologically, there was

definite hypertrophy of the adrenal cortex in the right adrenals of the animals in Group one, mild hypertrophy in those of Group two, and none in those of Group three. The good correlation between adrenal weight, histology and corticosteroid output is thus evident.

The administration of one international unit of ACTH failed to produce a significant rise in corticosteroid output from the left or right adrenals of any of the dogs. Since this dose of ACTH is more than sufficient to produce a maximal corticosteroid output in the normal dog (2) the stress of surgically exposing the adrenal gland and cannulating the adrenal vein was in all instances associated with the maximal 17-hydroxycorticosteroid output of which the gland was capable at the time the determination was made.

TABLE 1. ALL FIGURES ARE MEAN VALUES FOR SIX DOGS PLUS OR MINUS THE STANDARD ERROR OF THE MEAN

Group	17-hydroxycorticosteroid output (micrograms/min.)		Right adrenal weight (mg./kg. body weight
	Left adrenal	Right adrenal	
Group 1. (Left adrenal cannulated and removed one week before right cannulated)	7.2±0.8	10.2±0.8	76.6±0.8
Group 2. (Left adrenal cannulated but left in place one week before right cannulated)	7.2±0.6	6.4±0.5	62.9±1.0
Group 3. (Left adrenal removed two hours before right cannulated)	5.9±0.8	4.6±0.8	55.9±1.0

DISCUSSION

The present data show that there is a significant increase in the maximum 17-hydroxycorticosteroid output from the remaining adrenal gland one week after unilateral adrenalectomy. This is correlated with an increase in the weight of the gland and histologic evidence of adrenocortical hypertrophy. That this rise is dependent on removal of one adrenal is shown by the fact that it does not occur after sham adrenalectomy. That it requires time to develop and is not an immediate consequence of adrenalectomy is shown by the fact that it did not occur when the right adrenal vein was cannulated two hours after left adrenalectomy.

Because all output values in this study are from maximally stimulated adrenal glands, no conclusions can be drawn about the sensitivity of these glands to very small doses of ACTH. This problem has been under study in this laboratory. In three dogs, repeated small doses of ACTH injected 4–6 hours after hypophysectomy produced the same corticoid output from one adrenal before and after the opposite adrenal was acutely removed. On the other hand, preliminary results appear to indicate that dogs unilaterally adrenalectomized 1–2 weeks before hypophysectomy show a greater 17-hydroxycorticoid output per unit of ACTH from the remaining adrenal than do dogs with both adrenals intact.

SUMMARY

The 17-hydroxycorticosteroid output from the adrenal gland in the dog was studied in three groups of animals. In one group, the left adrenal vein was cannulated, samples of adrenal venous blood obtained, and the left adrenal gland then removed. One week later, the right adrenal vein was cannulated. In a second group, the left adrenal vein was cannulated but the left adrenal was not removed, and one week later the right adrenal vein was cannulated. In a third group of animals, left adrenal vein cannulation and left adrenalectomy was performed and samples of venous blood from the right adrenal were obtained two hours later. It was found that unilateral adrenalectomy was not associated with an immediate change in the output from the remaining gland, but that one week after unilateral adrenalectomy, corticosteroid output from the remaining adrenal was significantly increased when compared to the output from the remaining gland one week after sham adrenalectomy. The right adrenal glands removed from the animals subjected to left adrenalectomy one week previously were heavier than those obtained from the animals subjected to sham adrenalectomy, and showed definite evidence of cortical hypertrophy. There was a good correlation between mean adrenal weight, histologic appearance, and mean corticosteroid output for the three groups of dogs.

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THE EFFECT OF ANDROGENS ON THE OVARIES AND UTERUS OF THE ESTROGEN TREATED HYPOPHYSECTOMIZED IMMATURE RAT

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ESTROGENS quantitatively stimulate the immature ovarian follicles of hypophysectomized immature rats resulting in the production of medium-sized solid follicles (1); while testosterone propionate given with estradiol benzoate to similar animals causes degenerative changes in the ovarian follicle and its egg (2).

Development of a fluid-distended thin-walled uterus by estrogens and of a heavy muscular uterus following androgen administration is well known. Recent studies describing the effect of various combinations of steroids on uterine weight in hypophysectomized immature rats have revealed interesting anomalies in the actions of certain of these compounds (3).

The present investigation is concerned with quantitative aspects of estrogen-androgen interaction and with comparison of several androgenic steroids upon the diethylstilbestrol stimulated ovaries and uteri of hypophysectomized immature rats. The relative "anti-estrogenic" potency of several androgens has been compared by several routes of administration.

METHODS

Female rats of the Holtzman strain, 24–25 days of age weighing 40–50 gm., were hypophysectomized by the parapharyngeal route. Treatment was begun immediately after operation and continued daily for 4 days. The animals were killed by cervical fracture 48 hours after the last injection. Ovaries were dissected free of adventitious tissue and weighed without delay. The uteri were stripped of mesometrium, drained of excess fluid and blotted before weighing.

The compounds to be studied were dissolved in sesame oil or prepared as aqueous suspensions in concentrations calculated to provide the daily dose in a volume of 0.1–0.2 ml. The estrogen-treated animals were injected subcutaneously with 1.0 mg. of diethylstilbestrol daily for 4 days. When two compounds were given concurrently they were administered at different sites.

Ovaries and uteri were fixed in 10% buffered formalin. Histological preparations were stained by the hematoxylin-eosin method.

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RESULTS

Effect of androgens on the maximally estrogen stimulated ovaries of the hypophysectomized immature rat

When increasing doses, from .04 through 4.0 mg., of testosterone propionate were injected subcutaneously a graded decrease in ovarian weight occurred in the diethylstilbestrol enlarged ovaries (Table 1, Fig. 1).

Microscopic examination of ovaries from those hypophysectomized animals who had received only diethylstilbestrol revealed uniform medium-sized solid follicles with normal ova (1). These ovaries bore no evidence of

TABLE 1. EFFECT OF TESTOSTERONE PROPIONATE* ON THE OVARIES AND UTERUS OF THE HYPOPHYSECTOMIZED IMMATURE RAT

Total dose testosterone propionate, mg.	No. of animals	Mean ovarian wt., mg.†	No. of animals	Mean uterine wt., mg.†
With 4 mg. Diethylstilbestrol:*				
40.0	10	10.03 ± .63	10	108 ± 4
20.0	2	9.82	—	—
4.0	13	10.02 ± .51	11	108 ± 4
1.2	2	11.58	2	112
0.4	22	13.71 ± .76	21	101 ± 3
0.04	12	16.19 ± .90[a]	12	104 ± 4
0.004	14	22.51 ± 1.61	10	103 ± 6
—	17	24.37 ± .71	15	114 ± 4
No Diethylstilbestrol:				
40.0	8	7.07 ± .74	8	142 ± 4[b]
4.0	5	6.55 ± .54	5	122 ± 2
0.4	9	9.19 ± .84	9	108 ± 4
0.04	8	8.31 ± .38	8	51 ± 3
0.004	8	9.33 ± .44[c]	8	43 ± 7
—	16	7.91 ± .35	17	29 ± 5

* One quarter of total dose given daily for 4 days beginning immediately after hypophysectomy.
† ±Standard error.
[a] P <.01 vs T.P. .004 mg., +D.E.S. 4 mg.
[b] P <.01 vs T.P. 40 mg., +D.E.S. 4 mg.
[c] P <.025 vs control.

follicular atresia and numerous mitotic figures were present in their granulosa cells. Estrogen-stimulated ovaries were further characterized by a paucity of interstitial tissue, which appeared less vascular and more edematous than that of the hypophysectomized controls. The thecal cells were not grossly decreased in number but appeared thinner, and primordial follicles were only rarely seen.

When 0.004 mg. of testosterone propionate (T.P.) was given in combination with diethylstilbestrol (D.E.S.) the ovaries remained predominately of the estrogenized type. However, an occasional follicle could be seen undergoing what appeared to be early normal atresia around an intact ovum (Fig. 3). Increasing doses of T.P. induced progressively greater swelling and degenerative changes in the ova, cytolysis of the granulosa cells, vascularity of the theca, and decline in follicular size.

The largest follicles in the ovaries from animals receiving 40.0 mg. T.P. (and D.E.S.) were only about $\frac{1}{3}$ to $\frac{1}{4}$ the size of comparable ones in ovaries maximally stimulated by estrogen; while many primordial follicles were present, these, like the larger follicles, showed obvious degenerative changes (Fig. 6). Ovaries from those animals receiving the largest doses of T.P. with D.E.S. lacked intact ova, and contained severely pyknotic granulosa cells without mitotic figures, increased vascularity of the theca interna and in-

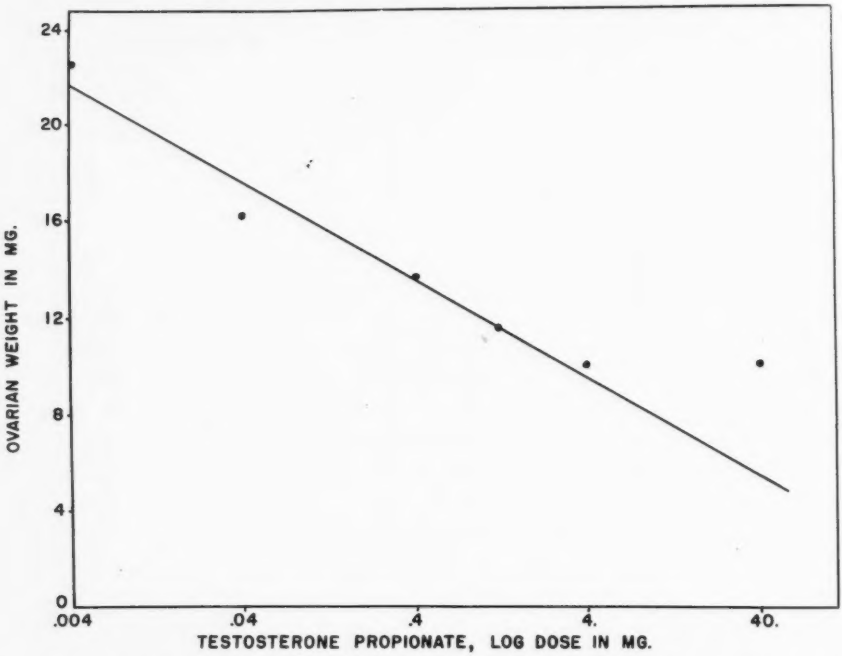


FIG. 1. The effect of various doses of testosterone propionate on ovarian weight in the diethylstilbestrol-treated hypophysectomized immature rat. The regression line was calculated by the method of least squares.

creased interstitial tissue. The latter was interspersed with abundant pink staining "hyaline-like" material and also exhibited increased vascularity and marked tortuosity of blood vessels. Thus, T.P. produced a graded change from the estrogen to the androgen modified type of ovary (Figs. 5 and 6). Failure of the largest dose of T.P. to further decrease ovarian weight to that of the non-treated hypophysectomized controls might be attributed to the increased production of ovarian interstitial tissue provided by this androgen.

The ability of various other androgens to limit weight increase of the estrogen-stimulated ovary was compared with that of testosterone propionate administered subcutaneously (Table 2). Methylandrostenediol, an-

drostane-17β-ol-3-one and 17α-ethyl-17 hydroxynorandrostenone were quantitatively comparable to testosterone propionate in the production of ovarian changes. Methyltestosterone, adrenosterone and 19-nortestosterone produced this response to a lesser degree and progesterone was without significant effects even in large doses. However, when 20 mg. progesterone was given with D.E.S. early follicular atresia, similar to that seen with 0.004 mg. of T.P., was present (comparable to Fig. 3).

The estrogen-inhibiting efficacy of androgens administered by various routes was investigated (Table 3). 17α-ethyl-17 hydroxynorandrostenone proved more potent than methyltestosterone or testosterone propionate following oral administration. Although testosterone propionate was considerably less effective by inunction than by the subcutaneous route it was more effective by this route than when given orally. No qualitative differences between the various androgens were brought out by the different routes of administration. A close correlation between microscopic appearance and change in gross ovarian weight was evident with all of the androgens tested.

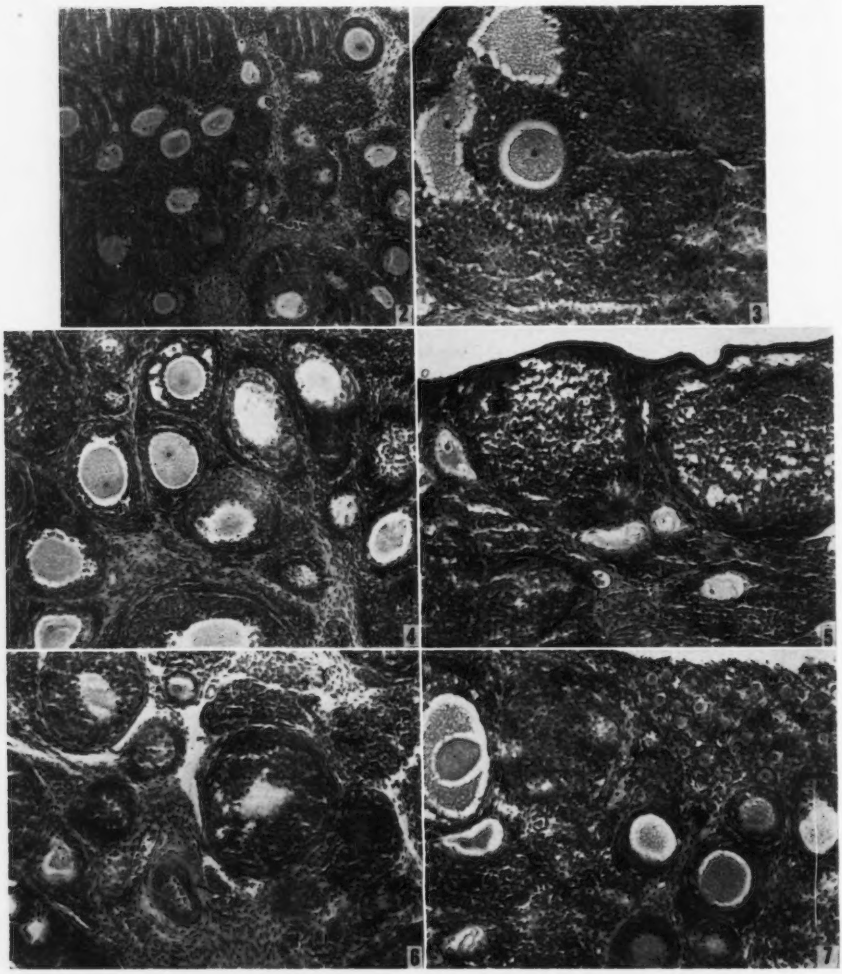
Ovarian effects of testosterone propionate when given alone to the hypophysectomized imature female rat

Testosterone propionate, at the smallest dose tested (0.004 mg.), caused degenerative changes in ova, cytolysis of granulosa cells and thickening of the theca interna (Fig. 4). Increasing doses of T.P. produced abundant primordial follicles, progressive degenerative changes of ova, more widespread necrosis of granulosa elements, thickening and more obvious vascularity of the theca and increasing cellularity and edema of the ovarian interstitial tissue. With large doses of T.P. (4.0–40.0 mg.), a characteristic microscopic picture of profound follicular dystrophy was seen, with increased interstitial "hyaline-like" material and a slight decrease in ovarian weight (Fig. 7, Table 1). Also, in the absence of administered estrogen, small doses of T.P. resulted in less follicular dystrophy and a slight increase in gross ovarian weight. This increase in ovarian weight appeared to be related to hyperplasia of the ovarian interstitial elements. Under the conditions of the present experiment no stimulation of the ovarian follicles by T.P. was observed.

Effects of androgens on the uterus of the hypophysectomized immature rat

Testosterone propionate produced no further uterine weight increase in hypophysectomized rats which had been maximally stimulated by diethylstilbestrol (Table 1). However, as the dose of T.P. was increased a progressive conversion of the thin-walled fluid-distended "estrogen type" uterus to the more compact non-distended "androgen type" was observed (Figs. 9, 10, and 11).

Upon microscopic examination, the uteri of the untreated hypophysec-



(FIGS. 2–7.) Ovaries from hypophysectomized immature rats, 120 hours post-operatively, treated with the stated daily dose of test material(s) given subcutaneously in oil daily for 4 days beginning immediately after hypophysectomy.

All magnifications ×100.

FIG. 2. Uninjected Controls. The print is slightly reduced in size.

FIG. 3. T.P. .001 mg.+D.E.S. 1 mg. There is uniform medium-sized solid follicle development characteristic of maximal estrogen effect. A follicle showing early atresia is illustrated.

FIG. 4. T.P. .001 mg. alone. The follicles are small and are characterized by a loose arrangement of granulosa cells leaving rather prominent "fluid spaces." There is early evidence of granulosa cell pyknosis and dissolution of ova; the latter effect being most evident in the primordial follicles.

FIG. 5. T.P. .1 mg and D.E.S. 1 mg. Follicles are smaller and more atretic than seen in Fig. 3 with further reduction in incidence of granulosa cell mitotic figures. There is in-

TABLE 2. INFLUENCE OF SEVERAL STEROIDS GIVEN SUBCUTANEOUSLY ON THE OVARIES AND UTERUS OF THE DIETHYLSTILBESTROL* TREATED HYPOPHYSECTOMIZED IMMATURE RAT

Steroid	Total dose, mg.**	No. of animals	Mean ovarian wt., mg.†	No. of animals	Mean uterine wt., mg.†
Diethylstilbestrol (Alone)	4.0	17	24.37 ± .71	15	114 ± 3
Methyltestosterone	0.4	12	19.20 ± 1.00	12	117 ± 2
Methylandrostenediol	4.0	3	10.8	—	—
	0.4	13	15.61 ± .82	14	119 ± 4
Androstane-17β-ol-3-one	20.0	4	14.73 ± 1.18	4	116
	0.4	12	15.88 ± .74[a]	12	111 ± 3
17α-Ethyl-17 hydroxy-nor-androstenone	0.4	10	13.82 ± .76	10	137 ± 4[b]
19-Nortestosterone	0.4	12	20.83 ± .78	11	117 ± 4
Adrenosterone	0.4	10	18.18 ± 1.06	10	121 ± 5
Progesterone	20.0	5	21.20 ± 1.82	2	131
	4.0	7	26.92 ± .60	5	116
Pregnenolone	20.0	2	33.68	4	116
Progesterone (Alone)	4.0	2	9.93	2	111

* 1 mg. daily for 4 days beginning immediately after hypophysectomy.
** One quarter of daily dose given daily for 4 days beginning immediately after hypophysectomy.
† ±Standard error.
[a] $P < .025$ vs 0.4 mg. Testosterone propionate + D.E.S. (Table 1).
[b] $P < .01$ vs 0.4 mg. Testosterone propionate + D.E.S. (Table 1).

tomized animals were found to have cuboidal endometrial epithelium, compact poorly vascular endometrial stroma with few or no mitotic figures and a slightly thin, but otherwise normal, myometrium (Fig. 8). D.E.S. stimulation of these animals caused uterine changes characterized by marked proliferation of tubular glands, a pseudo-stratified columnar type of endometrial epithelium, thinner and less cellular but more vascular stroma, and slight thickening of the myometrium (Fig. 8). T.P. in amounts from 0.4 through 40.0 mg. given with D.E.S. produced thickening of the uterine stroma with increasing cellularity, vascularity, edema and branching of glands. The latter uteri also exhibited increasing cell height of the endometrial epithelium and thickening of both myometrial layers though these effects were not striking (Figs. 10 and 11).

17α-ethyl-17 hydroxynorandrostenone proved more effective than T.P. in conversion of the D.E.S. uteri to the androgen-type. This steroid given

creased cellularity and vascularity of the interstitial tissue and more abundant "hyaline material" present. The theca interna appear vascular.

FIG. 6. T.P. 10 mg. + D.E.S. 1 mg. Widespread destruction of ova with severely pyknotic granulosa cells containing only rare mitotic figures and separated by prominent lacunae. The interstitial tissue is edematous and hyperemic with tortuous blood vessels and an abundance of pink staining "hyaline material." The theca interna is thin. The largest follicles are similar in size to those of the uninjected control animals.

FIG. 7. T.P. 10 mg. alone. The largest follicles are only about one-half the size of those in Fig. 2. Many primordial follicles are present though there is generalized destruction of ova. The interstitial tissue is abundant and hyperplastic. Thickening and increased vascularity of the theca interna is evident.

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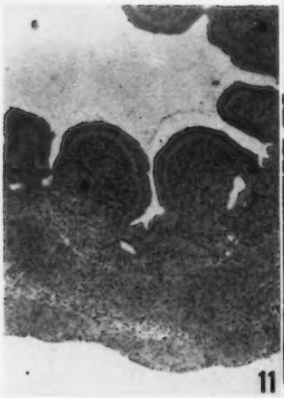
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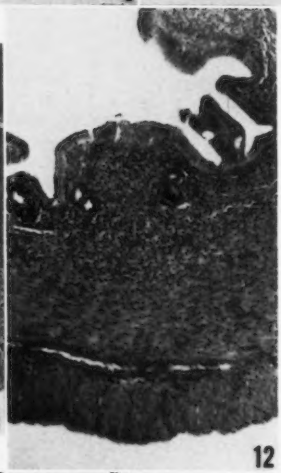
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TABLE 3. EFFECT OF SEVERAL ANDROGENS GIVEN BY VARIOUS ROUTES OF ADMINISTRATION ON THE OVARIES AND UTERUS OF THE DIETHYLSTILBESTROL* TREATED HYPOPHYSECTOMIZED IMMATURE RAT

Androgen	Total mg.**	Route of admin.	No. of animals	Mean ov. wt., mg.†	No. of animals	Mean uterine wt., mg.†
Testosterone propionate	40.0	Oral	11	21.86 ± 1.40	10	126 ± 6
Testosterone propionate	0.4	Oral	9	22.18 ± 1.24	9	121 ± 8
Testosterone propionate	40.0	Inunction	8	9.47 ± .58	8	89 ± 4
Testosterone propionate	4.0	Inunction	9	21.13 ± .97	9	113 ± 5
Testosterone propionate	0.4	Inunction	11	20.71 ± 1.15	8	109 ± 4
Methyltestosterone	4.0	Oral	11	21.90 ± .97	11	127 ± 3
Methyltestosterone	0.4	Oral	9	23.76 ± 1.02	9	108 ± 5
17α-Ethyl-17 hydroxy-norandrostenone	40.0	Oral	13	13.79 ± .56	13	157 ± 5[a]
17α-Ethyl-17 hydroxy-norandrostenone	4.0	Oral	10	18.93 ± 1.27	11	134 ± 4[b]

* 1 mg. daily for 4 days beginning immediately after hypophysectomy.
** One quarter of total dose given daily for 4 days beginning immediately after hypophysectomy.
† ± Standard error.
[a] $P < .01$ vs Testosterone propionate 40 mg. orally.
[b] $P > .05$ vs Testosterone propionate 40 mg. orally.

(FIGS. 8–14.) Uteri from hypophysectomized immature rats, 120 hours post-operatively, treated with stated daily dose of test material(s) given subcutaneously in oil daily for 4 days beginning immediately after hypophysectomy. All magnifications ×30.

FIG. 8. Uninjected control. The endometrial epithelium is cuboidal and shows no evidence of secretoary activity. No mitotic figures are present in the rather compact endometrial stroma. The myometrium also appears dense and highly cellular.

FIG. 9. D.E.S. 1 mg. alone. Pronounced distention of the uterine cavity with marked proliferation of tubular endometrial glands. The endometrial epithelium is pseudostratified columnar in type. Endometrial stroma is less cellular, showing mild edema and increased vascularity, measuring only about ¼ the thickness seen in Fig. 8. The myometrium is slightly thicker than seen in Fig. 8.

FIG. 10. T.P. 10 mg. + D.E.S. 1 mg. Small uterine cavity with minimal branching of glands. The pseudostratified columnar epithelium is slightly taller than in Fig. 9 with midzone nuclei; there is no clear evidence of secretory activity. No mitotic figures are present in the endometrial stroma and moderate edema is present between the stroma and myometrium. The myometrium is of approximately the same thickness as in Fig. 9 but appears more compact.

FIG. 11. T.P. .1 mg. + D.E.S. 1 mg. Moderate uterine cavity formation with abundant proliferation of glands. The endometrial stroma is similar to Fig. 9 but slightly thicker. Myometrial thickening with a rather loose arrangement of cells is present.

FIG. 12. 17α-ethyl-17 hydroxynorandrostenone .1 mg. + D.E.S. 1 mg. (Comparison to Fig. 11.) The cells of the endometrial epithelium appear more "secretory." The endometrial stroma is thicker and more edematous with abundant tortuous glands, and the myometrium is thicker.

FIG. 13. T.P. 10 mg. alone. The uterine cavity is small and filled with abundant mucoid material. Papillary hyperplasia is evident in the endometrium. Endometrial epithelium cells are cuboidal in type with basal nuclei, their cytoplasm containing secretory granules which stain basophilic. A few mitotic figures are present in the endometrial stroma which also shows marginal edema.

FIG. 14. T.P. .1 mg. alone. (In comparison to Fig. 13.) Less mucoid material is present in the glands. The endometrial epithelial cells appear less "secretory."

either subcutaneously or orally induced weight increments in the maximally D.E.S.-stimulated hypophysectomized rat uterus (Tables 2 and 3), an effect not seen with the other androgens used. A total of 0.4 mg. of this compound given subcutaneously produced greater branching of the endometrial glands, a more hyperplastic stroma and greater thickness of the myometrium than the largest dose of T.P. used. The latter effects appeared similar to that seen in the progesterone-D.E.S. uteri (Fig. 12).

The histological effects of other androgens were compared upon uteri maximally stimulated by diethylstilbestrol. With the exception of 17α-ethyl-17 hydroxynorandrostenone, all appeared less effective in the production of progestation-like endometrial changes and muscular increase than comparable doses of testosterone propionate.

T.P. given by inunction in large doses (40.0 mg. total) caused a decrease in D.E.S.-induced uterine weight (Table 3).

When T.P. alone was administered to hypophysectomized immature rats in ascending doses a progressive increase in uterine weight resulted (Table 1) which at the maximum dose given (40.0 mg.) exceeded that found with concurrent administration of D.E.S. Histologically these uteri showed somewhat more progestation-like activity than their T.P.-D.E.S. counterparts, with more abundant mucoid material appearing in the endometrial glands, (Figs. 13 and 14).

DISCUSSION

It must be borne in mind that the data presented are concerned only with hypophysectomized weanling rats given generally intensive estrogen and androgen treatment for a relatively short period of time.

The production of degenerative changes in ova, particularly in the smaller follicles, appears to be an early effect of androgens as suggested by Gaarenstroom and DeJongh (2). The quantitative decrease in ovarian weight and graded degeneration of follicular elements, serve as a means of comparing various androgens by different routes of administration. The effects observed in this study could be due to androgenic, anti-estrogenic or other activities of certain steroids presently classified as androgens.

No clear evidence of ovarian follicle stimulation was seen with the doses of testosterone tested whether the animals were estrogen-treated or not, although this effect has been described in the intact rat by Noble (4). It has further been demonstrated by Gaarenstroom and DeJongh (2) that anterior pituitary secretions protect against the destructive effect of androgens on the ovum and its follicle. Small doses of testosterone given alone to hypophysectomized rats slightly increased ovarian weight probably due to proliferation of ovarian stromal elements accompanied by comparatively less follicular dystrophy.

Testosterone propionate when given alone to the test rats produced a slight but graded increase in progestational characteristics of the uterine

epithelium as well as an increase in uterine weight. Diethylstilbestrol was in some respects antagonistic to this effect, reducing uterine weight increase and progestational development. The compound 17α-ethyl-17 hydroxynorandrostenone, given either orally or subcutaneously, was less inhibited by D.E.S. It thus seems evident that this steroid exerted considerably more progestational effect, or was less modified by estrogens in its effect on the uterus, than the other androgens tested.

Testosterone propionate administered in large doses by inunction decreased the weight of uteri maximally stimulated by estrogen. As this effect was less evident after subcutaneous administration of testosterone propionate the possibility of its resulting from intermediates formed on passage through the skin might be considered. In accord with this possibility would be the finding of Huggins and Jensen (3) that other steroids of the androstane series (and progesterone) are more powerful than testosterone in inhibiting estrone-stimulated uterine growth. Estrogen, at least in large doses, prevented further increase in uterine mass even upon heavy androgen stimulation though these uteri were changed grossly and histologically to the androgen-type.

Estriol inhibition of estrone stimulation of the rat uterus, first described by Hisaw, and collaborators (5), occurs even when large doses of these compounds are given together (1). Generally diethylstilbestrol, which is a strong uterine growth stimulant, appears to inhibit the powerful uterine growth promotion which ordinarily results from large doses of testosterone propionate given subcutaneously. 17α-ethyl-17 hydroxynorandrostenone however, shows the singular property among the androgenic steroids tested of causing further growth in the already maximally estrogen-stimulated uterus of the hypophysectomized immature rat. This is of further interest in view of the finding of Huggins and Jensen (6) that progesterone itself is a strong inhibitor of estrone-induced growth of the rat uterus under similar circumstances.

Estrogen, in large doses, induces uniform growth of ovarian follicles to the medium-sized stage and then appears to maintain these follicles and their eggs in a viable state. It would further appear that estrogens play some part in slowing the formation of new primordial follicles after the medium-sized solid follicle stage is reached as well as in providing some measure of protection for the larger follicles.

Further advance in follicular size is largely due to accumulation of intrafollicular fluid accompanied by progressive disintegration of the follicular granulosa cells and wholesale premature atresia of remaining intermediate follicles and their eggs. The remarkable processes of growth and destruction serving to concentrate the energies of the ovary toward rapid mobilization of the surviving ova could be, in some measure, provided by androgen.

Gaarenstroom and DeJongh (2) have suggested that androgen produced by the interstitial cells of the ovary is normally responsible for follicular

atresia (if one can assume that androgen is normally produced by the ovary (7, 8)) and that protection of the disinvesting ovum is provided by anterior pituitary secretions. Upon the basis of the experiments here described it might appear that some portion of this protective effect could be attributed to the relatively high titers of estrogen which are produced during this stage of follicular development.

Facilitation of ovulation and stimulation of luteal cells can be produced by administration of small doses of testosterone to the intact animal, as described by Noble (6). The present experiments performed in the absence of the pituitary gland but in the presence of estrogen indicate that androgen in very small doses produces cavitation in advancing ovarian follicles, vascularization of the theca interna and destruction of ova in smaller follicles. These effects might be construed to serve a useful function in the ovarian cycle.

SUMMARY

Testosterone propionate, given subcutaneously, quantitatively inhibits the ovarian weight gain induced by a standard dose (4.0 mg.) of diethylstilbestrol in the hypophysectomized immature rat. Several androgenic steroids (testosterone propionate, 19-nortestosterone, 17α-ethyl-17 hydroxynorandrostenone, adrenosterone, methylandrostenediol, androstane 17β-ol-3-one and methyltestosterone) given by various routes were compared upon the basis of this effect. These androgens, in graded doses, produced quantitative deterioration of the ovarian follicle and its ovum, an effect somewhat modified by estrogen protection of the follicle. Testosterone propionate was found to be more efficiently absorbed by inunction than after oral administration.

Diethylstilbestrol partially inhibits the effect of androgen upon the uteri of hypophysectomized immature rats, restricting both weight increase and progestational changes.

Acknowledgments

We are indebted to Dr. I. C. Winter of G. D. Searle and Company for supplies of 17α-ethyl-17 hydroxynorandrostenone and to Dr. W. W. Byrnes of the Upjohn Company for 19-nortestosterone and adrenosterone.

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THE RELATION OF SUCCINATE TO ADRENOCORTICAL FUNCTION[1,2]

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EARLIER preliminary experiments have stimulated our curiosity and interest in the probable relationship between succinate and adrenocortical function during stress. It is well known that oxygen uptake is stimulated by succinate. We have seen that there is almost a typical dose-response curve regarding oxygen uptake when liver homogenates are treated with increasing doses of succinate (1). It is of interest, however, that the stimulating effect of succinate on oxygen uptake of liver homogenates may be greatly inhibited by cortisone. This is striking, since we have observed that cortisone itself, causes approximately a 21% increase of oxygen uptake above untreated controls (2). The question arose, therefore, as to the liklihood of cortisone acting to slow down the metabolic rate rather than stimulate it during stress. If our thesis is correct that succinate may cause a rapid turn-over of the metabolic reserve, while cortisone would slow down this increased turn-over, it would be important to demonstrate this on the basis of adaptation to stress conditions; that is, a succinate treated animal should show a shorter survival due to a rapid utilization of the metabolic reserve while cortisone would antagonize this effect resulting in increased survival. Also it would be of value to titrate the "succinate effect" with varying doses of cortisone to show the difference in survival of such animals exposed to cold stress. Similarly, the stimulative effect of succinate on oxygen uptake of liver homogenates should be titratable with varying doses of cortisone.

The present experiments are designed to demonstrate the antagonistic action of cortisone against succinate in both *in vitro* and *in vivo* set-ups.

METHODS

1. *Oxygen Uptake Experiments*

In this set of experiments, liver tissue from normal male Sprague-Dawley rats was used. The animals were sacrificed by decapitation and the liver was rapidly removed and placed on ice. Four hundred mg. samples of liver were used throughout the study. For the succinate (alone) experiments, the tissue was treated with 20 mg. of sodium succinate

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[2] The Authors express thanks to Merck and Company for the Cortisone used in this study.

prior to homogenization. The tissue was then homogenized in phosphate buffer (pH 7.45). In the succinate-cortisone group, the same amount of sodium succinate was used, but doses of cortisone ranging from 1 to 10 mg. were used. These samples were also homogenized in phosphate buffer. Following homogenization the liver samples were transferred to Warburg flasks, which were placed in a water bath at 37.5° Centigrade and oxygenated for 15 seconds. The flasks were then equilibrated for 10 minutes, following which uptake of oxygen was measured in three 10 minute periods. Liver tissue untreated with succinate or cortisone constituted the untreated controls, which were run with each set of experiments. The results are expressed in percent change from the controls.

2. *Stress Experiments*

Male mice, weighing 10 to 12 grams (NIH General Purpose Strain) were used throughout this study. Intact and adrenalectomized mice were administered 20 mg. quantities of sodium succinate intraperitoneally dissolved in phosphate buffer (pH 7.45). Controls were treated with phosphate buffer, using the same volume as in the experimental groups but containing no other substance. Cortisone titration experiments were set up, administering to groups of succinate-treated animals doses of cortisone varying from 0.1 mg. to 1.0 mg. Both intact and adrenalectomized animals were used in these experiments. All animals were placed in a cold room (5° C) approximately a half hour after injections. The animals were allowed to remain in the cold until they no longer survived. The cold room was checked every half hour for surviving animals. Percentage survival was tabulated following each group of experiments. Groups of mice of the same weight as above were treated with sodium succinate and allowed to remain at room temperature in order to determine whether or not succinate itself was deleterious.

RESULTS

Figure 1 shows the results of O_2 uptake studies titrating the succinate with varying doses of cortisone. Results are expressed in percent increase

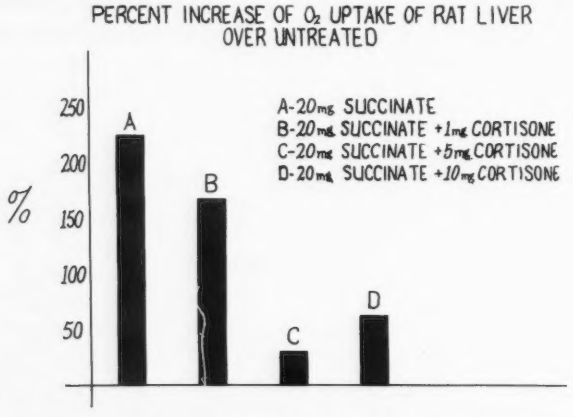

FIG. 1. Showing the effect of succinate on the O_2 uptake of liver homogenates and the antagonism of the "succinate effect" by cortisone. There were ten or more experiments in each group. Each group was compared with the untreated controls and showed greater than 99% confidence. Controls showed mean of 26.5 (S.E. .76) micro liters of O_2 uptake per gram tissue homogenate.

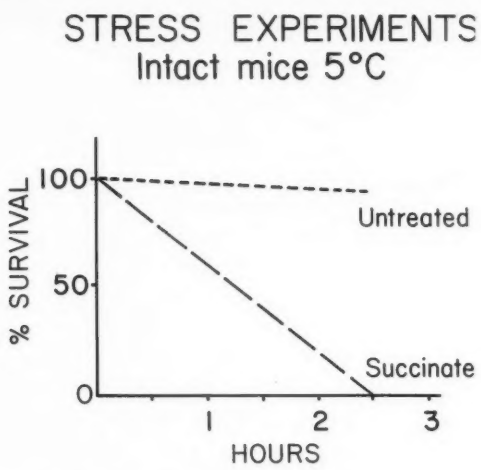

FIG. 2. Showing the effect of succinate on the survival of intact mice exposed to cold (5° C). Treated and untreated groups contained thirty animals each (P = .01).

over controls. Controls showed average O_2 uptake of 26.5 micro liters per gram of tissue (S.E. .76). It can be seen that the stimulating effect of succinate on oxygen uptake is markedly lowered by cortisone. It appears that 5 mg. quantities of cortisone were more effective than any dose in antagonizing the effect of succinate.

Figure 2 shows the effect of succinate on survival of intact mice. It can be seen that all succinate treated animals had succumbed to the cold stress after two and a half hours, while there was approximately 98% survival of

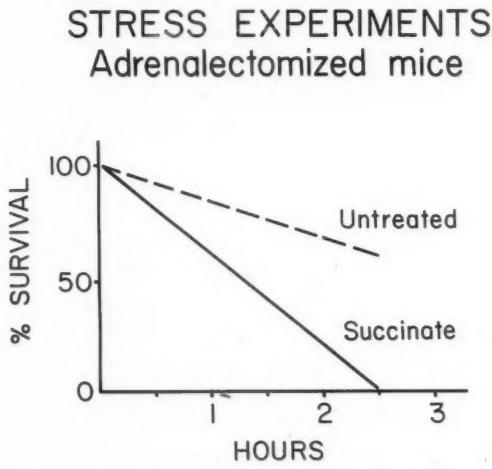

FIG. 3. Showing the effect of succinate on the survival of adrenalectomized mice in cold (5° C). There were twenty animals in each group (P = .03).

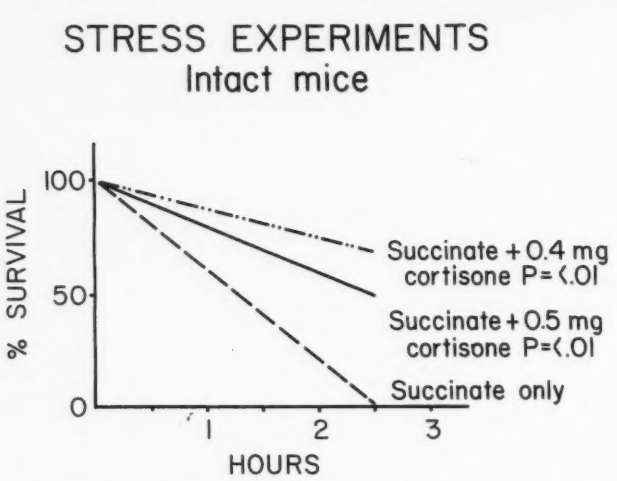

FIG. 4. Showing the effect of cortisone when used against succinate in intact mice (there were twenty animals in each group). All animals were exposed to cold (5° C).

the control untreated animals. Mice injected with sodium succinate and left at room temperature showed no deaths. In Figure 3 data are presented showing the influence of succinate on the survival of adrenalectomized mice. The effect on the adrenalectomized animals is essentially the same as on intact animals. Unlike the intact controls, however, the adrenalectomized controls showed a considerable decrease in survival which would be expected.

Figure 4 shows the effect of cortisone on succinate-treated intact animals

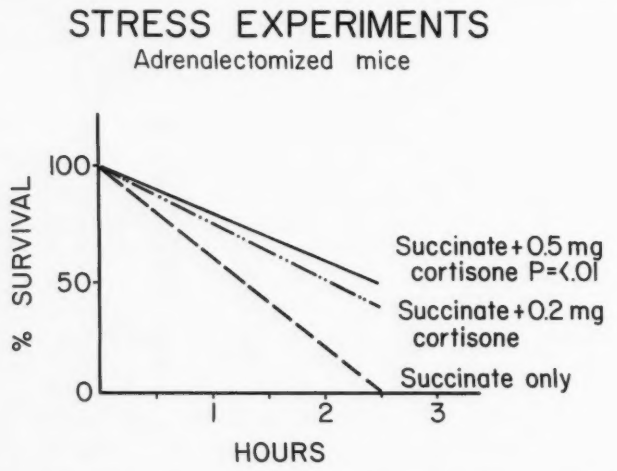

FIG. 5. Showing the antagonistic effect of cortisone in adrenalectomized mice exposed to cold (5° C). There were twenty animals in each group.

when subjected to cold stress. The 0.4 mg. dose was more effective than the 0.5 mg. dose. Both doses, however, reduced markedly the effect of succinate in cold. The 0.4 mgm. dose of cortisone in succinate treated animals showed a survival of 75%, while the 0.5 mg. dose of cortisone in succinate gave a survival of 60% in 2½ hour periods. All animals treated with succinate alone were dead at the end of this experimental period. In a few experiments we tried higher doses (1 to 5 mg.) of cortisone than are reported here, but it was observed that the survival was poorer in animals so treated than in the succinate group alone. Figure 5 shows the effect of cortisone on the succinate-treated adrenalectomized animals. Although the survival is not as good in this group as in the intact group, it is clear that cortisone antagonized the "succinate effect." Both the 0.2 mg. and the 0.5 mg. doses gave greater than 50% survival at the end of 2½ hours, while all succinate-treated (alone) animals had died.

DISCUSSION

Our results indicate that succinate is deleterious to the adaptation of animals to cold stress and that this effect may be antagonized by cortisone in the whole animal, both intact as well as adrenalectomized. The results with animals exposed to stress are in agreement with the liver oxygen uptake studies. In both types of experiments cortisone acted to antagonize the effect of succinate. Weiss and Moss (3) have shown that liver homogenates of stressed animals (rats) increase in their oxygen uptake following stress. You and Sellers (4) have shown that the succinoxidase system of certain tissues of stressed animals is increased during exposure to cold stress. This may indicate that succinate is developing at a rapid rate during stress. The poor survival of succinate-treated animals in cold stress suggests that adaptation to cold stress is impeded by succinate. We have been attracted by the recent work of Bacchus and Lampkin (5) who have shown the influence of certain members of the citric acid cycle on the metabolism of cortisone. These investigators have shown that when oxalacetate, fumerate, malate and succinate were added to separate preparations of liver slices, the rate of metabolism of cortisone was increased above the normal untreated liver slices; but citrate, alpha ketogluterate and pyruvate failed to influence the metabolism of cortisone. The results with citrate interest us for the reason this substance failed to cause oxygen uptake stimulation of liver homogenates even in the presence of TPN or DPN. It may be possible to postulate that those members of the citric acid cycle which hasten the metabolism of cortisone may not favor adaptation. If this is tenable, it is conceivable that succinate, when administered would result in shortened survival during cold stress. The fact that varying doses of cortisone have different effects on succinate treated animals suggests a quantitative relationship regarding the antagonistic effect between succinate and cortisone. When doses of cortisone outside of the "permissive" range were used

against succinate, there was diminution of survival below that of animals treated with succinate alone. Likewise, in the oxygen uptake experiments, when the most effective dose of cortisone was reached, increasing the dose resulted in a greater oxygen uptake rather than a diminished oxygen uptake. It is of interest in this connection that no dose of cortisone was found in the oxygen uptake experiments that would depress the succinate-influenced oxygen uptake to a level equal to that of untreated controls.

SUMMARY

Experiments have been cited showing the stimulation of oxygen uptake of liver tissue by succinate and showing that succinate-treated animals show a poor adaptation to cold stress. In both the *in vitro* and *in vivo* experiments the "succinate effect" was antagonized by cortisone in proper dosage. The suggestion was offered that cortisone may act during stress to slow down the demand upon the metabolic reserve which succinate would seem to draw upon.

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THYROXINE SECRETION IN RATS EXPOSED TO COLD[1]

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INTRODUCTION

THE importance of the thyroid hormone in the increased heat production necessary for survival in a cold environment has been established in the rat (1). Several indices, including histological changes (2, 3, 4), the effects of anti-thyroid substances (5, 6) and iodine metabolism (7, 8) indicate that thyroid activity increases shortly after exposure to cold and remains high for some time. However, whether a high level of thyroid activity continues throughout prolonged cold exposure has been questioned.

In various studies, values obtained by several indirect indices of thyroid activity have returned to, or toward normal after about 30 or more days' cold exposure. For example, Starr and Roskelley (4) noted that the height of the thyroid epithelium was at, or near, control levels in rats which had lived 45 to 56 days at 12°–17° C. Pichotka (9) found maximum thyroid weights in guinea pigs exposed to 0–3° C at 4 weeks of exposure; the weights in animals living 10–14 weeks at this temperature corresponded to control weights. The histological picture, however, still strongly suggested increased activity (9, 10). Radio-isotope studies in the rat suggest a change in thyroid activity after more prolonged cold exposures. At 40 days at 0°–4° C, thyroid radioiodine content 2 hours after injection had decreased to levels similar to those in control animals (7, 8). Thyroid radioiodine release likewise decreased (7).

The over-all metabolism during cold exposure follows a pattern similar to that of the thyroid. Schwabe, Emery and Griffith (11) kept rats at 8°–12° C, 16 hours per day for 14, 30 and 60 days; oxygen consumption measured at 29° C in exposed animals remained elevated 10–15% over controls regardless of duration of exposure. Metabolism, as estimated from oxygen consumption measurements at 1.5° and 30° C, remained elevated throughout exposure in Sellers and You's (12) study of animals living at 1.5° C up to 90 days. A decrease in oxygen consumption between 45 and 90 days' exposure was indicated by the measurements at 1.5° C, but not those at 30° C. Cottle and Carlson (13), measuring heat production directly at 5° C in animals kept at 5° C up to 60 days, recorded a gradual decline in metabo-

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lism between 35 and 60 days. At this time, heat production at 25° C was not significantly different in control and in cold-exposed rats.

Although these indices of thyroid function can be interpreted to indicate some decrease in thyroid activity during prolonged cold exposure, none are direct enough to be conclusive. Certainly iodine metabolism is modified; however, the over-all effect may well be a complete adaptation of the pituitary-thyroid system to different and more efficient plane. Accordingly, the effect of prolonged cold exposure on thyroid activity has been investigated by the more direct method of thyroxine replacement. In this study, thyroxine secretion in animals exposed to cold 60 days has been estimated by two methods: (a) the amount of exogenous thyroxine needed to prevent propylthiouracil-induced thyroid hypertrophy, and (b) the amount of thyroxine required to elevate oxygen consumption to normal in thyroidectomized rats.

METHODS

Adult male Sprague-Dawley rats, weighing 280–380 gm., were placed in individual metal cages. Control animals were kept in a laboratory at 25° C ± 1°. Cold-exposed animals were kept in a cold room at 5° C ± 1°. Thyroxine requirement was determined by two independent methods.

Experiment I. Thyroxine secretion was estimated by a method similar to that described by Dempsey and Astwood (6). Groups of 25 animals were kept in the cold (5° C) for periods of 14, 30 and 60 days. Twenty animals of comparable age, kept at 25° C, served as untreated controls for groups in the cold. All animals were given Purina Chow and water *ad libitum*. Fourteen days prior to sacrifice, all animals of each group, except those serving as untreated controls were given 0.1% propylthiouracil (PTU) with the Purina Chow. Graded doses of d,l-thyroxine diluted to a volume of 0.2 ml. were administered daily by subcutaneous injection to animals receiving PTU; untreated control animals received normal saline injections. By this means, gradations in thyroid hypertrophy (i.e., thyroid weight) were obtained. After 14 days, the animals were sacrificed and the thyroid glands were removed, weighed on a Smith-Roller torsion balance to the nearest 0.2 mg.

Experiment II. Groups of 25 rats were kept at 5° C for 14 and 60 days; 20 comparable animals were kept at 25° C as controls for both groups in the cold. Five or six animals in each group were kept intact and fed Purina Chow and water *ad libitum*. The remaining animals were thyroidectomized under ether anesthesia 2 weeks prior to metabolism measurements. Calcium lactate 1% was added to drinking water of thyroidectomized animals during these 2 weeks. Thyroidectomized rats were fed the iodine-deficient diet described by Remington (14) and modified by Leblond and Eartly (15) to contain an estimated 10 to 100 parts iodine per billion. L-thyroxine in graded doses was injected subcutaneously into thyroidectomized rats in a volume of 0.2 ml., and saline administered to the controls.

Oxygen uptake was measured in a closed-circuit apparatus at 25° C. After 20 minutes' equilibration, oxygen consumption was measured over a half-hour period. Oxygen contained in spirometers calibrated in cubic centimeters of gas per centimeter of drop was circulated at a constant rate of 4 L/min/animal by air pumps. Soda lime in 1-inch brass cylinders removed carbon dioxide. All determinations were made at 25° C; temperature was kept constant by immersing the cylinders in a thermostatically regulated water bath. Temperature and pressure were corrected to standard conditions. Reading-

were taken exactly 10 minutes apart directly from a centimeter scale on the side of each spirometer. The first two readings were disregarded because the animals were active during much of this time. Thereafter, the average of the smallest three consecutive 10-minute oxygen consumptions in the next 50-minute period were considered the half-hour basal oxygen uptake for each rat. Results are expressed in cubic centimeters of oxygen consumed per hour per kilogram$^{3/4}$ body weight. Duplicate recordings on each animal were made on successive days.

To ascertain whether thyroidectomy was complete, the animals were sacrificed several days following recordings, and thyroid tissue was sought in the tracheal region. Suspect tissue was fixed and examined. Data from animals in which thyroid tissue was found were disregarded.

RESULTS

Experiment I. The size of daily doses of exogenous d,l-thyroxine required to reduce to normal the thyroid gland weight in PTU-treated animals was estimated from the data presented in Table 1. Thyroid weight varied in-

TABLE 1. THYROID GLAND WEIGHTS
(Mg. per 100 gm. body wt.)

Treatment	14 days at 25° C		30 days at 25° C		58 days at 25° C	
	No. of animals	Weight ±SD	No. of animals	Weight ±SD	No. of animals	Weight ±SD
None	4	4.25±0.34	4	4.44±0.30	5	5.25±1.15
PTU+1 μg. thyroxin/day	3	18.41	—	—	—	—
PTU+3 μg. thyroxin/day	4	16.33±1.84	4	14.91±0.96	4	13.85±1.92
PTU+5 μg. thyroxin/day	4	9.53±2.06	4	13.23±2.71	—	—
PTU+6 μg. thyroxin/day	—	—	—	—	4	8.07±1.59
PTU+7½ μg. thyroxin/day	4	5.72±1.25	4	9.73±1.04	—	—
PTU+10 μg. thyroxin/day	—	—	4	5.69±1.57	4	5.10±0.61

Treatment	14 days at 5° C		30 days at 5° C		58 days at 5° C	
	No. of animals	Weight ±SD	No. of animals	Weight ±SD	No. of animals	Weight ±SD
None	5	5.63±0.52	5	5.85±0.95	4	5.96±0.53
PTU+3 μg. thyroxin/day	6	18.19±2.36	—	—	—	—
PTU+5 μg. thyroxin/day	5	16.71±2.78	4	16.99±3.85	4	15.67±1.78
PTU+7½ μg. thyroxin/day	5	15.35±3.11	5	12.29±2.37	4	10.89±0.70
PTU+10 μg. thyroxin/day	5	9.74±1.16	5	9.99±2.60	5	10.19±1.20
PTU+15 μg. thyroxin/day	5	7.65±0.57	5	7.51±1.34	5	7.23±1.51

versely with dose of thyroxine in all groups of rats treated 2 weeks with PTU. The required daily dose of thyroxine was estimated from a plot of thyroxine dose versus the reciprocal of thyroid weight. The intersect of the best straight line with the mean thyroid weight of untreated control animals at 25° C was taken as the thyroxine requirement. An example is shown in Figure 1. Thyroxine requirement measured in this manner was more than double the control values in all groups exposed to 5° C whether for 14, 30 or 60 days. The thyroid weights of untreated rats increased significantly after 14 or 30 days at 5° C. After 60 days, the mean thyroid weight was

still high, but the difference between cold and warm was no longer significant. These findings substantiate previous reports in that thyroid hypertrophy, as measured by thyroid weight, was no longer demonstrable after prolonged cold exposure.

Experiment II. The amount of endogenous l-thyroxine necessary to maintain the oxygen consumption at control level was estimated from the data as illustrated in Figure 2. The oxygen consumption varied linearly with the dose of thyroxine. In this study a statistically significant differ-

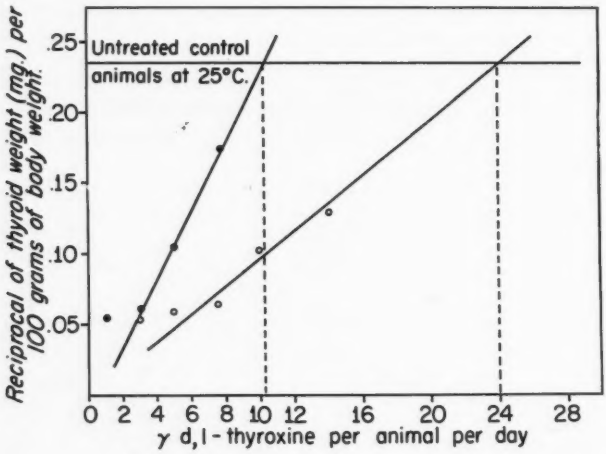
Reciprocal of thyroid weight (mg.) per 100 grams of body weight.
.25
.20
.15
.10
.05
Untreated control
animals at 25°C.
0 2 4 6 8 10 12 16 20 24 28
γ d,l - thyroxine per animal per day

FIG. 1. Thyroxine requirements of rats kept at 5° C for 14 days and of comparable animals at 25° C, measured by thyroid weight changes in propylthiouracil-treated animals. ● represents reciprocals of mean thyroid weight of animals kept at 25° C receiving PTU and various doses of d, l-thyroxine. ○ represents rats at 5° C. Thyroxine requirements of animals at 5° C is estimated to be 24 μg. per day; animals at 25° C require 10 μg. per day.

ence in oxygen uptake ($P < 5\%$) has been observed between the rats at any two dose levels in all groups, both in cold and in warm.

When measured at 25° C, oxygen uptake by intact animals increased by about 38% after 14 days at 5° C. It remained elevated to a similar level throughout the entire 60-day exposure (Table 2). This response was similar to that reported by Schwabe *et al.*, (11) and Sellers and You (12). Similarly, where the same dose of thyroxine had been administered to comparable sets of animals at 25° C and 5° C the basal oxygen uptake of the cold-exposed animals was in each case significantly higher than that of animals kept in normal temperatures, both at 14 and at 60 days.

The daily dose of l-thyroxine required to raise the oxygen uptake of thyroidectomized rats to the level of that of intact control animals at 14 days was 3.5 μg. at 25° C and 7.5 μg. at 5° C; at 60 days the daily dose of l-thy-

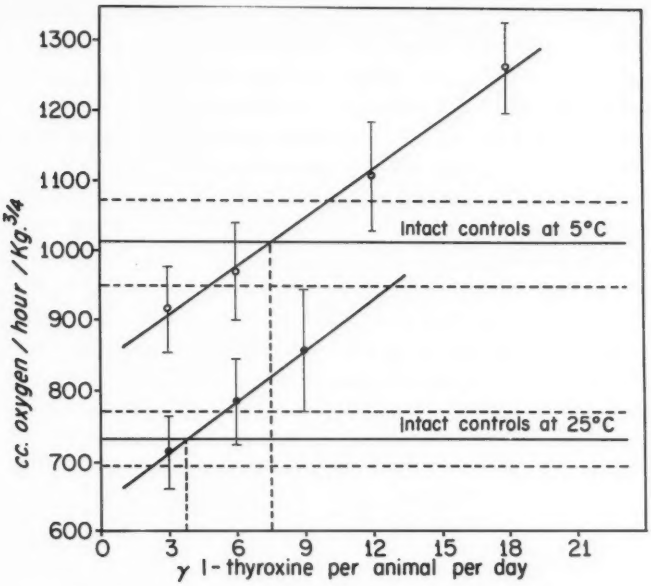

FIG. 2. Thyroxine requirements of animals kept for 14 days at 5° C and of comparable animals at 25° C, measured by oxygen uptake response to thyroxine by thyroidectomized rats. ● represents animals at 25° C; ○ represents animals at 5° C. Oxygen consumptions plus or minus one standard deviation are presented. Animals at 5° C require 7.5 μg. l-thyroxine per day; animals at 25° C require 3.5 μg. per day.

roxine was 5.0 μg. at 25° C and 12.0 μg. at 5° C. This index, also demonstrates that the need for thyroxine was more than doubled by exposure to 5° C for 14 or 60 days.

DISCUSSION

The two procedures used to determine daily thyroxine requirement in rats essentially confirm one another. It was found that 10–13 μg. of d,l-thyroxine per day prevents propylthiouracil-induced thyroid hypertrophy in 280–380 gm. rats kept at 25° C, while 3.5–5 μg. of l-thyroxine per day

TABLE 2. OXYGEN CONSUMPTION OF INTACT CONTROL RATS KEPT AT 5° AND 25° C MEASURED AT 25° C

Days of exposure	25° C		5° C		Per cent increase
	No. of rats	cc. O_2/kg.$^{3/4}$/hr.	No. of rats	cc. O_2/kg.$^{3/4}$/hr.	
4	6	742 ± 50	6	865 ± 33	17%
7	5	740 ± 39	5	924 ± 45	25%
14	10	731 ± 40	10	1011 ± 61	38%
36	15	830 ± 70	15	1078 ± 77	30%
54	6	759 ± 47	6	1006 ± 71	33%
60	6	750 ± 50	11	1023 ± 64	36%

elevates metabolism of thyroidectomized rats to normal. Part of the observed difference in dose requirement is due to the use of d,l-thyroxine in the first experiment. Barker, Kiely and Lipner (16) found greater oxygen consumption response to thyroxine in thyroidectomized rats than in PTU-treated animals. Similar variation is observed in thyroxine requirements of animals kept at 5° C, when the two procedures are compared, but the percentage increase in the requirement of cold-exposed animals is the same by either procedure.

The amount of exogenous d,l-thyroxine necessary to prevent hypertrophy of the thyroid gland in PTU-treated rats in effect agrees with the results of Dempsey and Astwood (6). Those workers found that 90–140 gm. rats required 5 μg. d,l-thyroxine when kept at 25° C, and about 10 μg. after 2 weeks at 1° C. The difference in ages and weights of the animals used in these studies accounts for the apparent difference in thyroxine requirements. Indeed, when thyroxine need is calculated on a basis of body weight, the results of the two studies are remarkably similar. About 30–40 μg., d,l-thyroxine per kilogram body weight per day are required at 25° C, and 75–85 μg. per kilogram are required after 14 days at 1–5° C.

To relate the requirement for exogenous thyroxine to physiological thyroxine secretion, it must be assumed that a fairly physiological dose of thyroxine administered daily in a single injection is metabolized and excreted by the animal in the same manner as the continually secreted endogenous hormone. Recent studies employing I^{131}-labeled thyroxine have shown that intact animals excrete parenterally administered thyroxine very rapidly in bile and urine. Gross and Leblond (17) followed the distribution of 0.001 to 0.007 μg. thyroxine-I^{131}, tracer amounts, which do not significantly elevate the plasma iodine level, and found the biological half-life to be only 18–24 hours. Larger doses of thyroxine, 0.7 to 20 μg. per animal, are distributed similarly to body tissues and appear to be excreted only slightly more rapidly than the very minute doses (17, 18, 19, 20). Klitgaard (21) using 90 μg. of labeled thyroxine per animal, found that biliary excretion of thyroxine decreases significantly in thyroidectomized and PTU-treated animals, but that urinary excretion is little affected. Taurog (20), however, finds little change in biliary excretion on injected thyroxine-I^{131} by athyroid animals. It thus seems valid to assume that a series of fairly physiologic doses of thyroxine administered daily to PTU-treated and surgically thyroidectomized rats are metabolized similarly to the endogenous hormone in intact animals. It has been found that a given dose of thyroxine-I^{131} leaves the plasma and is excreted more rapidly in cold-exposed than in normal animals (unpublished data). The increase in thyroxine secretion and utilization in cold exposure may thus be somewhat less than the estimated 110–140%; nevertheless, the increase appears to be the same in all durations of exposure.

The data obtained in these studies, then, seem to indicate markedly in-

creased secretion and utilization of thyroxine in animals exposed to cold, even for prolonged periods. The amount of exogenous thyroxine required to restore metabolism and thyroid weight to normal in athyroid rats is more than doubled in rats exposed to 5° C, whether for 14, 30 or 60 days. This indicates that the mechanisms responsible for thyroid activation are still functioning as before, after prolonged cold exposure.

Support for the conclusions drawn in this investigation is found in work done simultaneously at this laboratory by Cottle and Carlson (22). They found the biological decay of injected I^{131} to be accelerated in animals exposed to cold for periods up to 60 days. Similarly, conversion of I^{131} to thyroxine-I^{131}, as measured by the serum inorganic I^{131}; serum PBI^{131} ratio following I^{131} injection, is more rapid and more complete in animals kept at 5° C up to 2 months than in controls. These findings further establish the continued increase in thyroxine secretion and metabolism of prolonged cold exposure.

SUMMARY

Thyroxine secretion in animals exposed to cold for various durations has been estimated by two approaches. (a) The amount of d,l-thyroxine required daily by cold-exposed rats to prevent the thyroid hypertrophy of propylthiouracil treatment has been determined. This is an estimate of the thyroxine needed to restore the hypophyseal-thyroid system to its normal equilibrium. (b) The daily dose of l-thyroxine which restores oxygen consumption of thyroidectomized rats to normal has been estimated. This is a measure of the over-all peripheral action of that hormone.

Results of both studies confirm the previous evidence that thyroxine secretion is greatly increased following 2 weeks at a low environmental temperature. It is further established that thyroid activity remains at a high level throughout a 60-day cold exposure.

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SOME QUANTITATIVE AND QUALITATIVE ASPECTS OF I^{131} METABOLISM IN TURTLES[1]

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ALTHOUGH the reptile has received but little attention in studies of thyroid physiology, it has been shown that the reptile thyroid gland 1) contains iodine (1), 2) contains a substance similar to thyroxine (2), 3) gives a typical histological response to thiouracil and thyrotropin (3, 4, 5, 6). However, the functional significance of thyroid hormone in reptiles remains in doubt (7). It is the purpose of the present report to present data on the metabolism of radioiodine by the turtle.

MATERIALS AND METHODS

Two species of adult turtles were utilized, a terrestrial species, *Terrapene carolina*, and an aquatic species, *Pseudemys floridanus*. The *Terrapene* were collected in the vicinity of Brookhaven National Laboratory during the months of June and July, while the *Pseudemys* were purchased from a commercial source and it was not certain that they were truly representative of the season in which they arrived at the laboratory. The complete protocol as to the environmental temperature, access to water, and the administration of thyrotropin is given in Table 1. Fifteen to 40 μc. of I^{131} as carrier-free iodide was injected by the intraperitoneal route. Measurement of quantitative uptake of I^{131}[6] was done by either of two techniques: 1) *In vivo;* periodic measurement of radioactivity over the thyroid area of individual turtles was done by use of an external collimated Geiger counter. 2) *In vitro;* tissues to be assayed for radioactivity were removed at sacrifice from groups of turtles at regular intervals following I^{131} administration. In both cases an aliquot of the original injection solution was counted under similar conditions and the results are expressed as the percentage of the administered isotope found in the sample. Selected thyroid glands from both groups were prepared for histological study while various tissues other than thyroid glands were assayed for radioactivity.

Chromatographic analysis of the chemical forms of the thyroidal iodine from glands studied by the *in vitro* method was done by a technique closely following that of Lissitzky (8). Briefly, weighed samples of tissue were ground in an all-glass homogenizer, and digested for 48 hours with pancreatin at pH 8.5 followed by 24 hours of papain

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[6] Obtained from Oak Ridge National Laboratory.

digestion at pH 5.3. The hydrolyzed mixture was then extracted with acidified butanol and the extract evaporated to dryness at temperatures maintained below 45° C. The concentrated extractable iodine compounds were applied to filter paper for descending chromatography by butanol-acetic acid and butanol-ammonia solvents. For identification of radioactive spots, known monoiodotyrosine (MIT), diiodotyrosine (DIT), potassium iodide (KI), triiodothyronine (TITN), and thyroxine (TX) were chromatogrammed in parallel with the hydrolyzed thyroidal material, or as carriers of it. Before beginning any experiment, the animals were allowed to become accustomed to the conditions of the experiment, i.e., temperature, access to water, for 7–10 days. In all cases the animals were assigned to experimental groups on the basis of body weight and sex. When thyrotropin was given, each animal received a single daily intraperitoneal injection of 1.0 mg. of thyrotropin,[7] beginning 7 days before administration of I^{131} and continuing until sacrifice.

The general protocol and results are presented in Table 1.

TABLE 1. GENERAL PROTOCOL AND RESULTS

Genus	Experimental manipulation	Month used	Number of animals used	I^{131} administered, $\mu c.$	Temperature, °C	Availability of water	Maximum uptake	Hours at which compound appeared			
								MIT	DIT	Tx	Titn
Terrapene	Effect of cold and/or TSH on uptake of radioiodine	Aug	12	20	21–23 2– 3	Dry Dry	80 25	Not done			
Terrapene	Effect of wet environment	Aug.	2	40	21–23	Dry Wet	70 15	Not done			
Terrapene	Sacrificed in groups of 5 at 6–264 hours after isotope administration	July	30	15	18–24	Wet	25	6	6	52	
Pseudemys	Sacrificed in groups of 4 at 24–96 hours after isotope administration	Mar.	28	15	21	Dry	80	24	24	none	none
Pseudemys	Effect of cold, sacrificed in groups of 2 as above	Mar.	10	15	10	Dry	15	Not done			
Pseudemys	Sacrificed in groups of 6 at 1–27 days after isotope administration	Sept.	30	20	21	Dry	15	24	72	72	144

RESULTS

The thyroidal accumulation of I^{131}, determined in *Terrapene* by the *in vivo* method, of animals placed in the cold was less than of control animals. The administration of thyrotropin (TSH) was effective in increasing the rate of thyroidal accumulation, and possibly of the release of I^{131}, only in animals maintained at the control temperature. The small sample size and individual variation noted in these results permits no correlation of functional findings and morphological findings although it does appear that female terrapins were somewhat less responsive than their male counterparts (Fig. 1). Similar results were noted in *Pseudemys*, when studied in March by the *in vitro* method under dry conditions although when *Pseudemys* was studied in September the accumulation values were smaller than those noted in March (Fig. 3). When the terrapins were maintained in a wet en-

[7] Thyrotropin, beef, Lot No. R317-147, contributed by Armour Laboratories, Chicago.

vironment, less I^{131} was accumulated by the thyroid and more I^{131} was excreted than when similar studies were done in a dry environment (Fig. 2, Table 1).

Various tissues from *Pseudemys* were further examined for radioactivity. Although most tissues contained little I^{131}, it was noted the urine contained most of the extra-thyroidal I^{131} (Table 2). In general, as the amount of I^{131} found in the thyroid gland increased the amount of I^{131} in the urine decreased.

Three chromatographic analyses were made of thyroid gland tissue from

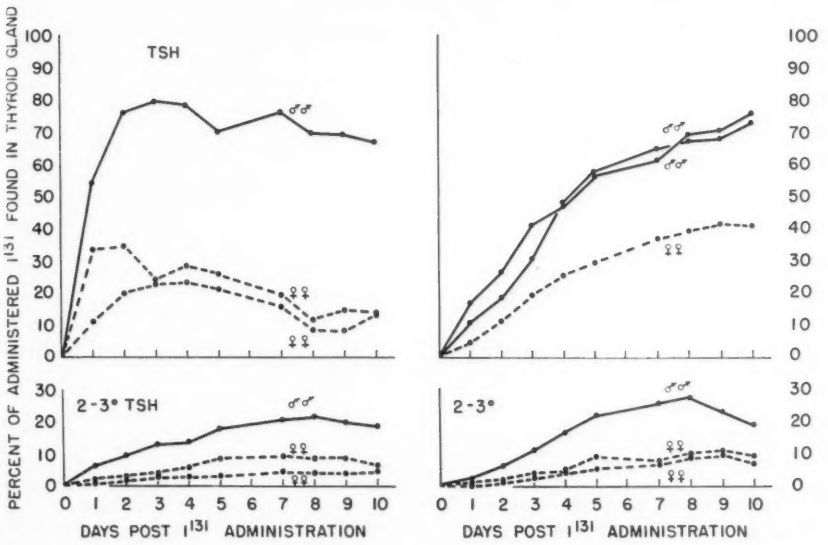

FIG. 1. The per cent of administered I^{131} found in the thyroid gland plotted against days between isotope administration and measurement. Determined by the *in vivo* method in dry, August, *Terrapene carolina*, with and without TSH administration. The upper curves were plotted from animals maintained at 21–23° C, the lower curves obtained from animals maintained at 2–3° C. TSH refers to the administration of thyroid stimulating hormone (see text).

Pseudemys and *Terrapene* at selected intervals following the administration of I^{131} (Fig. 3, Table 1). When glands from warm, dry, *Pseudemys* were studied in March, at 1 and 4 days following radioiodine administration, only monoiodotyrosine and diiodotyrosine were found. However, when the same species was studied in September under similar experimental conditions, in addition to the presence of radioactive monoiodotyrosine and diiodotyrosine, labelled thyroxine was noted as soon as 3 days after isotope administration and the amount of thyroxine appeared to increase throughout the 27-day study. Triiodothyronine, in an extremely small amount, was detected only at 6 days following I^{131} administration. In the case of

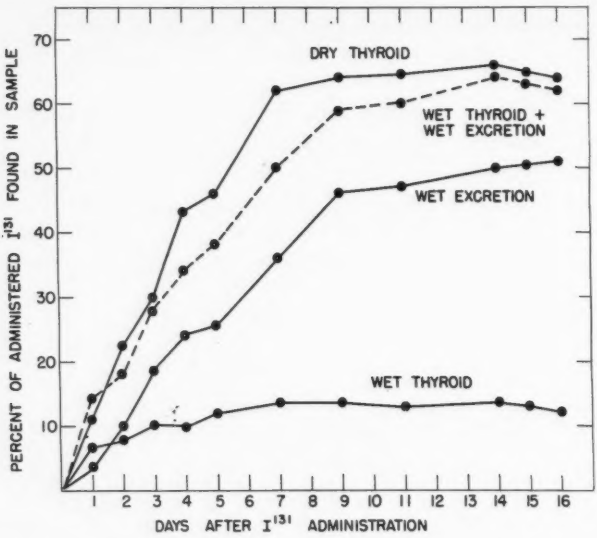

FIG. 2. The per cent of administered I^{131} found in the thyroid gland, recovered urine, and gland plus urine, of a *Terrapene carolina* maintained under wet conditions and the per cent of administered I^{131} found in the thyroid gland of a dry *Terrapene carolina*, plotted against days between isotope administration and measurement. Both animals maintained at 21–23° C and studied in August (males).

Terrapene, studied in July under wet conditions, in addition to labelled monoiodotyrosine and diiodotyrosine, labelled thyroxine was noted at 52 hours following I^{131} administration. The amount of thyroxine appeared to increase until the experiment was terminated at 264 hours while triiodothyronine was not definitely identified.

The histological appearance of the glands ranged from inactive in warm,

TABLE 2. DISTRIBUTION OF INJECTED I^{131} IN INDIVIDUAL PSEUDEMYS (SEPTEMBER, 21° C)
See Figure 3, curve 3

Days post injection	% of I^{131} in thyroid	% of I^{131} in bladder	% of I^{131} in 1.0 ml. blood	% of I^{131} in 1.0 gram of muscle
3	1.9	92.5	0.004	0.001
3	2.1	81.1	.009	.001
3	19.8	60.2	.011	.008
6	1.8	58.7	.002	.001
6	2.3	70.3	.008	.001
6	8.9	58.7	.012	.001
14	5.3	52.7	Trace	Trace
14	7.2	47.6	Trace	Trace
14	17.9	58.4	Trace	Trace
27	4.6	63.1	Trace	Trace
27	6.5	49.0	Trace	Trace
27	9.6	74.0	Trace	Trace
27	19.7	41.4	Trace	Trace

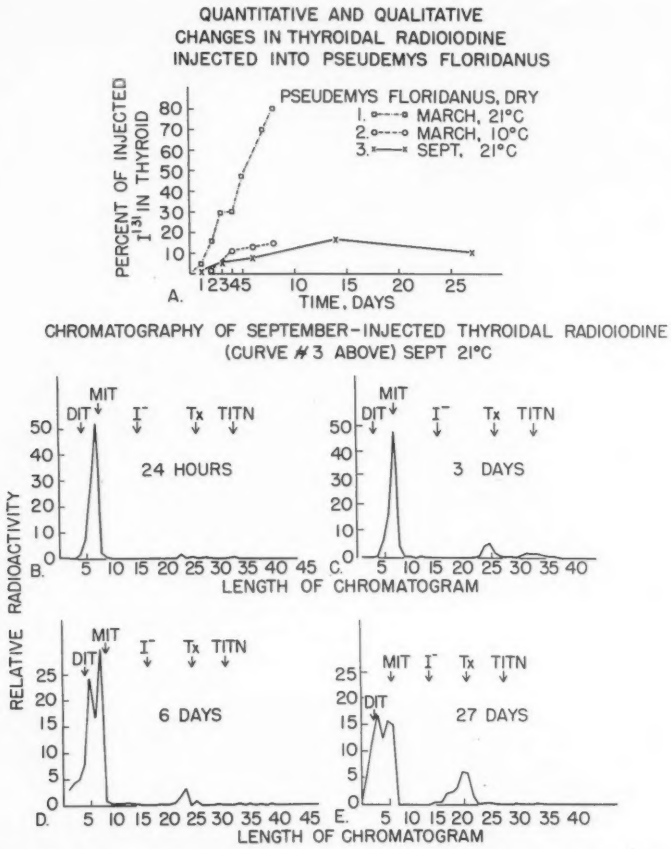

FIG. 3. The percent of administered I^{131} found in the thyroid gland of *Pseudemys floridans* plotted against days between isotope administration and sacrifice, and the results of chromatographic analysis of these glands.

dry, September *Pseudemys* through active in warm, wet, July *Terrapene*, with the warm, dry, March *Pseudemys* being some intermediate (Figs. 4, 5).

DISCUSSION

The present experiments have demonstrated that the reptilian thyroid gland has the capacity to accumulate radioactive iodine, to synthesize thyroxine and possibly triiodothyronine, and to respond to thyrotropin by increasing the rate at which I^{131} is accumulated. In these respects the function of the thyroid gland resembles the function of the gland in other vertebrates.

The present finding of a direct relationship between environmental

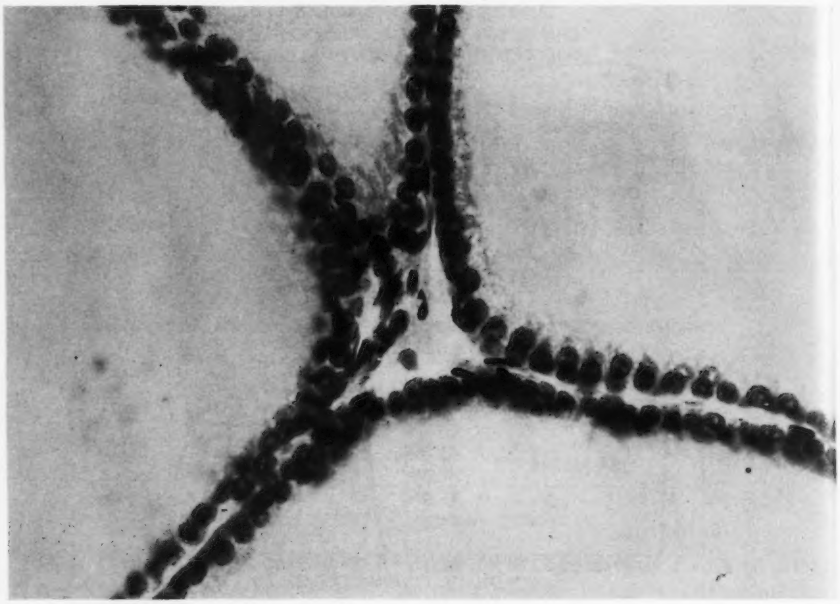

FIG. 4. Photomicrograph of thyroid gland of September *Pseudemys*. Bouin fixation, hematoxylin and eosin staining (×400).

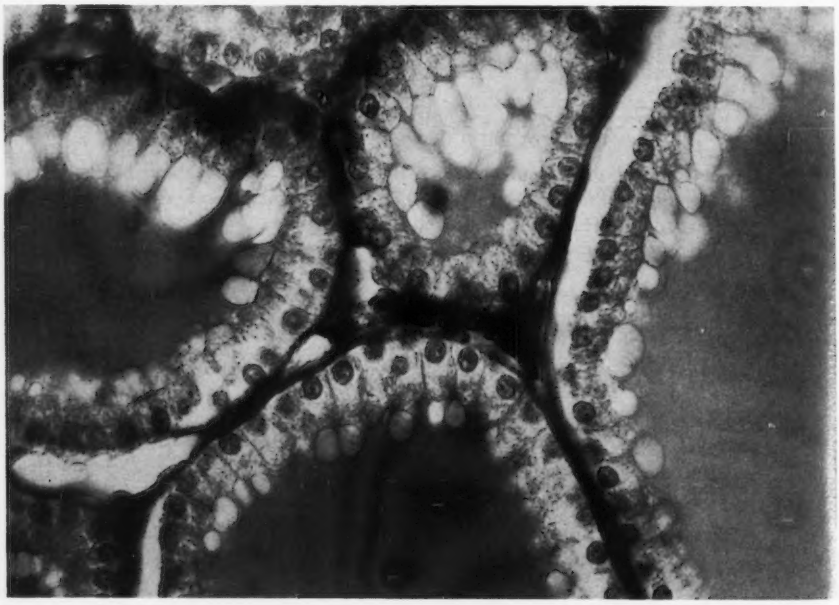

FIG. 5. Photomicrograph of thyroid gland of July *Terrapene*. Bouin fixation, hematoxylin and eosin staining (×500).

temperature and thyroid function is in agreement with similar studies done in fish (9, 10). It would appear that these two-cold-blooded species exhibit a direct relationship between environmental temperature and thyroid function while warm-blooded animals exhibit an inverse relationship between thyroid function and environmental temperature (11, 12, 13, 14, 15, 16). However, when the temperature of the thyroid gland of warm-blooded animals was lowered below a certain threshold, by induced hypothermia or hibernation as done by Ariel and Warren (17) and Deane and Lyman (18) in rabbits and hamsters, thyroid function was depressed. Thus, there may be a threshold of temperature below which the thyroid gland no longer functions. This suggestion receives support from the experiments of Freinkel and Ingbar (19) who noted that rabbit thyroid slices maintained *in vitro* at temperatures below 26° C no longer had the capacity to accumulate I^{131}. The inability of thyrotropin to influence the rate of thyroidal accumulation of I^{131} when turtles were kept at 2–3° C, while the same amount of hormone increased the rate of thyroidal accumulation of radioiodine when the animals were maintained at warmer temperatures, may likewise be due to a temperature dependent situation, *i.e.*, as the temperature is lowered the response of the thyroid gland to thyrotropin is reduced.

Certain of the thyroidal accumulation curves of I^{131} of the turtles maintained under dry conditions were remarkable since they indicated that I^{131} entered the thyroid gland at a slow rate but that a maximum uptake of approximately 80% of the administered dose was in the gland 8 days after administration while the turtles maintained under wet conditions accumulated much less radioiodine. It was thought that the higher thyroidal uptake in dry turtles might be a reflection of decreased renal excretion of iodide since a preliminary search of the cages that housed the dry turtles revealed little or no radioactivity while the water containing the wet turtles showed the presence of much radioactivity. Since samples of blood from the dry turtles contained only traces of radioiodine even a few hours after I^{131} administration a systematic search of tissues for radioactivity was begun and it was noted that the urine in the urinary bladder of *Pseudemys* contained a large portion of the injected radioiodine. Although there was some variation in the I^{131} content of the bladder and urine, in one case they accounted for more than 90% of the injected radioiodine. This mechanism of urinary retention, which possibly plays a role in resistance to desiccation during dry periods, might serve as a source of material that is to be resorbed. It is well to remember that reptiles have a cloaca and the urinary bladder must empty into the posterior end of the gastro-intestinal tract. If reabsorption of iodide does not occur directly through the wall of the bladder, the posterior portion of the intestine may supply an alternative mechanism of reabsorption from the bladder. Thus, since a few preliminary tests suggested that the radioiodine in the urine was in the form of iodide, it seems apparent that a high proportion of the radioiodine was returned to

the circulation from the urinary tract to be metabolized by the thyroid gland.

The results of the chromatographic analysis of the thyroid glands were difficult to correlate with the thyroidal accumulation of I^{131} or the histological appearance of the glands. *Terrapene*, when maintained at warm temperatures with access to water and studied in July, and *Pseudemys*, when maintained at warm temperatures with no access to water and studied in September, both synthesized thyroxine, and the latter triiodothyronine, within the first 4 days following I^{131} administration, although the maximum uptake of radioiodine was only 15–25%. *Pseudemys*, when maintained at warm temperatures with no access to water and studied in March, synthesized no thyroxine or triiodothyronine although the maximum uptake of radioiodine was approximately 80% of the administered dose. The turtles that synthesized thyroxine had thyroid glands that gave a histological picture ranging from an inactive to an active gland. Although there are seasonal factors that are known to influence the structure of the reptilian thyroid gland (5) the present results do not seem sufficiently clear cut to estimate the relative function of the thyroid gland at different seasons since such diverse factors as sex, temperature, and access to water were not sufficiently well controlled.

SUMMARY

The thyroidal accumulation and metabolism of I^{131} was studied in two species of turtles, *Terrapene carolina* and *Pseudemys floridanus*. No significant differences were noted between the two species.

When the turtles were maintained at 21° C under dry conditions, approximately 80% of the administered I^{131} was accumulated by the thyroid gland in 7–9 days. Little or no excretion of radioiodine was observed under these conditions and it was suggested that the urinary bladder serves as a reservoir for I^{131} and that the radioiodine in the bladder may be returned to the circulation by way of the cloaca. This in part may account for the high, slowly achieved thyroid content of I^{131}. When the animals were allowed access to a wet environment, less radioiodine was accumulated by the thyroid but more I^{131} was excreted.

Exposure to a cold environment resulted in a low thyroidal accumulation of I^{131}. Thyrotropin was not effective in preventing this effect of cold on the thyroid gland although thyrotropin enhanced the thyroidal accumulation of I^{131} when it was administered to turtles maintained in a warm environment. It was suggested that thyroid function and the response of the thyroid gland to thyrotropin may be directly related to the temperature of the thyroid gland.

The presence of thyroxine, and in some cases triiodothyronine, was noted on the chromatograms of glands taken from animals that exhibited low uptakes of iodine, while these same glands gave a histological appearance

that varied from inactive to active. The lack of correlation or in some cases the negative correlation between the capacity of the gland to accumulate radioiodine and the capacity of the gland to synthesize thyroid hormone makes any attempt to assess the seasonal control of thyroid function of little value.

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EFFECT OF GLUCAGON ON GLYCOGEN IN RATS AND RABBITS

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IN AN earlier study of the effect of daily glucagon administration on rats and rabbits, the most interesting result was a large increase in the concentration of liver glycogen in both species (1). This paper reports the results of experiments designed to study further the increased concentration of liver glycogen produced by daily glucagon administration.

Helmer and Root (2) have suggested that the response of the blood sugar to glucagon administration is dependent upon the amount of liver glycogen available. Chronic daily administration of glucagon should bring about an increased response to a given dose of glucagon if there is a correlation between liver glycogen stores and glucagon response. Such an increase was found to occur in rabbits treated twice daily with glucagon for a period of 3 months.

METHODS

Rats and rabbits were maintained in individual cages in air-conditioned animal quarters. Rabbits were fed rabbit pellets and water *ad libitum*. Rats were force-fed a modification of Ingle's medium carbohydrate diet (3) twice a day. Each feeding, after a preliminary period of adaptation, was 12.5 cc. in volume. Water was available *ad libitum*. After the rats had been maintained on full feeding for 5 to 8 days the experiments with glucagon were started.

The glucagon used, Lot 208-108B-284,[1] has about one-tenth the hyperglycemic activity of crystalline glucagon. Doses are expressed in terms of this amorphous material.

At the end of each experiment the animals were anesthetized with sodium secobarbital ("Sodium Seconal," Lilly), various organs were removed, weighed quickly, and frozen rapidly between slabs of dry ice. Pieces of frozen tissue were weighed rapidly, digested in hot 30% KOH, and glycogen was determined by the method of Good, Kramer and Somogyi (4) with glucose measured by the Hagedorn and Jensen method (5). In some experiments the trichloracetic acid soluble (TCA) glycogen was determined by the method of Bloom, Lewis, Schumpert and Shen (6).

RESULTS

1. *Rabbits*—Administration of glucagon, 1 mg. subcutaneously twice a day to two rabbits, increased the response to glucagon injection in both rabbits. Figure 1 shows graphically some of the responses to the morning injection produced in one of the rabbits during 96 days of treatment. In Table 2 are figures for the blood sugar response to 1 mg. of glucagon each

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[1] The glucagon used in this work was an early lot prepared by Dr. Alfred Staub of our Biochemistry Division.

Monday morning and to the afternoon injection on some of these days for the 10-week period of the experiment. Also in this Table are the results of tissue glycogen analysis of organs from these two animals and from two control rabbits that received saline injections subcutaneously twice daily. The rabbits were killed for tissue analysis when in a fed state, 17 hours after the last injection. The mean liver glycogen concentration for the

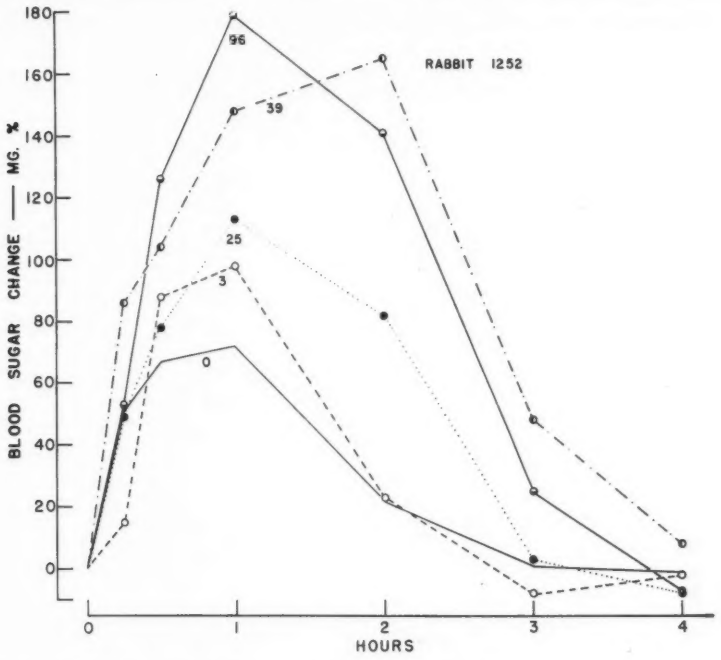

FIG. 1.—Blood sugar responses of one rabbit to morning injection of glucagon. The figure under each curve indicates the number of days of twice daily injections of glucagon, 1 mg. per injection.

glucagon-treated animals was 89.5 mg./gm. wet weight and for the saline controls, 45.6 mg./gm.

2. *Rats*—The effect of several variables on the liver glycogen response to the administration of 1 mg. of glucagon twice daily was studied in force-fed rats. The number of animals in each group varies and is given in Table 2. Table 2, Part A, is a summary of the results of experiments to determine the time course of development of increased liver glycogen in normal animals. Within three days there was a nearly tenfold increase in glycogen concentration in the glucagon-treated animals in one experiment. Continuing the glucagon injections for 7 to 14 days increased the liver glycogen concentration slightly, but the difference between 7 and 14 days was not significant.

The influence of various endocrine factors on liver glycogen stores is well known. In general, diabetic animals have low liver glycogen; adrenalectomized animals have nearly depleted glycogen stores; ACTH, cortisone and Insulin are all known to increase liver glycogen stores. When force-fed alloxan diabetic rats were injected twice daily with 1 mg. of glucagon, liver glycogen stores were more than doubled. Insulin treatment of similar animals (10 units of regular Insulin subcutaneously twice a day) also increased liver glycogen to values above normal (Table 2, Part B). However, in addi-

TABLE 1. THE EFFECT OF DAILY GLUCAGON ADMINISTRATION ON THE BLOOD SUGAR RESPONSE TO GLUCAGON INJECTION

(The first injection was given at 8 A.M. on 4–1–55)

Date	8 A.M.							4 P.M.						
	Control	15 min.	30 min.	1 hr.	2 hr.	3 hr.	4 hr.	Control	15 min.	30 min.	1 hr.	2 hr.	3 hr.	4 hr
						Rabbit 1252								
4- 1-55	98	149	165	170	120	99	97	109	144	142	144	108	107	93
4- 5-55	104	157	192	197	127	96	102							
4-12-55	99	156	185	200	111	97	86	103	162	177	180	116	103	87
4-19-55	88	156	193	185	112	79	80							
4-26-55	91	140	169	204	173	94	83							
5- 3-55	88	128	176	212	167	99	95							
5-10-55	90	176	194	238	255	138	98	94	129	174	159	115	110	94
5-24-55	91	125	180	201	174	104	101							
6- 7-55	111	167	218	258	204	130	109							
6-21-55	97	181	226	283	131	137	111							
6-28-55	84	129	174	209	106	90	81							
						Rabbit 1285								
4- 1-55	94	133	135	125	102	104	99							
4- 5-55	95	119	144	158	135	99	85	93	143	154	157	123	108	102
4-12-55	95	140	175	163	182	115	98							
4-19-55	85	143	185	199	127	102	76							
4-26-55	84	153	190	200	82	75	83							
5- 3-55	81	129	163	156	93	95	95	88	140	159	155	132	110	90
5-10-55	92	144	187	218	115	96	89							
5-24-55	105	152	189	184	101	99	91							
6- 7-55	116	197	204	219	139	112	113							
6-21-55	99	168	208	139	139	104	97							
6-28-55	86	157	198	95	99	79	79							
7- 6-55	96	164	202	200	109	88	99	112	147	156	129	127	121	114

Tissue	Glycogen—mg./gm. wet weight			
	Glucagon treated		Saline control	
	Rabbit 1252	Rabbit 1285	Rabbit 1253	Rabbit 1286
Liver	82.82	96.26	65.56	25.65
Heart	6.20	7.60	7.65	7.75

tion to increasing liver glycogen stores, Insulin also decreased urinary glucose excretion to zero, whereas the sugar excretion of glucagon-treated rats remained at the pre-treatment level (Table 4). In this particular experiment the untreated alloxan rats, although diabetic as indicated by substantial 24-hour urine sugar excretions (see Table 4), were able to maintain liver glycogen stores at the same level as that found in the normal control rats used in this experiment. A possible reason for this lack of difference in the two groups may be the fact that the diet used for force-feeding was apparently not adequate to maintain, even in normal rats, the high liver glycogen levels seen in rats fed *ad libitum* on a "chow" or "pellet" diet. In none of the experiments reported here was the liver glycogen concentration

of normal rats above 10 mg./gm. and in some it was as low as 2 mg./gm. In a group of six normal rats fed *ad libitum*, the liver glycogen averaged 51.3 mg./gm. and ranged from 45.9 to 58.3 mg./gm.

In another experiment, rats were regulated on the force-feeding regimen. They then were adrenalectomized and maintained with 1% saline to drink. Glucagon injections were started 48 hours after adrenalectomy and continued for one week. In Table 2, Part C, are the results of this experiment. Even in the absence of the adrenals, glucagon was capable of increasing liver glycogen stores nearly threefold.

TABLE 2. LIVER GLYCOGEN CONCENTRATION IN RATS
(Under treatment, numbers in parentheses indicate number of rats in group)

Treatment	Total glycogen mg./gm. ±S.E.	% increase	TCA extractable glycogen mg./gm.
Part A			
Normal-Saline treated—3 days (5)	3.33 ± 0.91		1.31
		732	
Normal-Glucagon treated—3 days (10)	27.69 ± 3.04		17.82
Normal-Saline treated—7 days (5)	2.15 ± 0.83		0.55
		1520	
Normal-Glucagon treated—7 days (10)	34.82 ± 2.11		32.22
Normal-Saline treated—7 days (5)	8.56 ± 2.05		4.30
		389	
Normal-Glucagon treated—7 days (9)	41.88 ± 4.66		27.57
Normal-Saline treated—14 days (5)	4.52 ± 1.29		2.18
		600	
Normal-Glucagon treated—14 days (9)	31.63 ± 4.91		20.57
Part B			
Normal-Saline treated (3)	9.97 ± 7.68		0.46
Normal-Glucagon treated (4)	68.90 ± 4.80		61.50
Alloxan diabetic-Saline treated (5)	9.79 ± 2.75		4.89
Alloxan diabetic-Glucagon treated (4)	23.24 ± 12.14		8.05
Alloxan diabetic-Insulin treated (4)	30.48 ± 7.05		17.96
Part C			
Normal-Saline treated (5)	5.44 ± 1.32		2.33
		504	
Normal-Glucagon treated (10)	27.42 ± 2.42		19.92
Adrenalex-Saline treated (4)	3.59 ± 2.45		1.66
		270	
Adrenalex-Glucagon treated (9)	9.71 ± 2.70		6.99
Part D			
Normal-Saline treated (9)	13.83 ± 2.93		8.59
		505	
Normal-Glucagon treated (8)	69.77 ± 6.37		59.33
Normal-Epinephrine treated (4)	2.72 ± 2.43		1.25

Epinephrine has been known to increase blood glucose by causing liver glycogenolysis and has been shown by Sutherland and Cori (7) to be as effective as glucagon in activating liver phosphorylase. If these actions of glucagon and epinephrine are identical and if the increase in liver glycogen

TABLE 3. LIVER GLYCOGEN CONCENTRATIONS OF RAT TISSUES AFTER TREATMENT WITH SALINE OR GLUCAGON

Treatment	Muscle, mg.%	Heart, mg.%	Kidney, mg.%
Normal-Saline—3 days	338	387	
		—P = >0.1	
Normal-Glucagon—3 days	354	428	
Normal-Saline—7 days	312	278	
		—P = >0.1	
Normal-Glucagon—7 days	321	348	
Normal-Saline—7 days	330	330	
		—P = >0.1	
Normal-Glucagon—7 days	277	421	
Normal-Saline—14 days	340	236	
		—P = 0.09	
Normal-Glucagon—14 days	296	360	
Normal-Saline	364		
Normal-Glucagon	434		
Alloxan-Saline	478		
	—P = 0.08		
Alloxan-Glucagon	356		
Alloxan-Insulin	434		
Normal-Saline	158	266	
		—P = 0.02	
Normal-Glucagon	168	392	
Adrenalex-Saline	159	239	
Adrenalex-Glucagon	158	228	
Normal-Saline	508	262	39.2
		—P = 0.03	—P = 0.04
Normal-Glucagon	531	359	46.5
Normal-Epinephrine	616	267	38.8

which we have observed involves the phosphorylase system in any way, epinephrine might also be expected to increase liver glycogen stores. Ten rats received 1 mg. of glucagon twice daily, ten rats received 30 μg. of epinephrine subcutaneously twice daily, and ten rats served as saline-treated controls. After seven days of treatment, the liver glycogen concen-

TABLE 4. MEAN 24-HOUR URINE SUGAR EXCRETION OF ALLOXAN DIABETIC RATS BEFORE AND DURING TREATMENT WITH GLUCAGON OR INSULIN
(Figures in parentheses indicate numbers of days in each period)

Rat No.	Urine sugar, gm./24 hrs.	
	Pre-treatment	During treatment
8	1.62 (32)	—
9	2.20 (32)	—
10	2.44 (32)	—
11	0	—
12	1.58 (32)	—
13	2.73 (6)	2.84 (5) HGF, 1 mg. 2×/day
14	2.10 (7)	2.22 (21) HGF, 1 mg. 2 ×/day
15	0.83 (12)	1.25 (19) HGF, 1 mg. 2 ×/day
16	2.44 (12)	2.96 (20) HGF, 1 mg. 2 ×/day
17	2.70 (8)	2.65 (21) HGF, 1 mg. 2 ×/day
18	0	0 Insulin, 5 units 2 ×/day
19	2.59 (12)	After 3 days became neg. (20) Insulin, 10 units 2 ×/day
20	2.01 (6)	Neg. Insulin, 10 units 2 ×/day
21	2.81 (1)	0.30 (19) Insulin, 12 units 2 ×/day

tration of the glucagon-treated animals was 5 times that of the control rats, whereas the epinephrine-treated animals had liver glycogen stores which were depleted to near starvation levels (Table 2, Part D).

In some of these experiments, the liver glycogen concentration was studied in organs other than the liver. Table 3 presents the results of all these studies. In no case did glucagon alter the glycogen stores in striated muscle, heart, or kidney from that found in comparable control rats.

DISCUSSION

The results of this and previous experiments with the chronic administration of glucagon in large doses to rats and rabbits show that this substance is capable of causing glyconeogenesis (1). The fact that the sensitivity of rabbits treated daily with glucagon is enhanced, strengthens the suggestion (2) that the status of liver glycogen stores determines the degree of hyperglycemia produced by a standard dose of glucagon. The lack of an enhancement of the afternoon injection of glucagon is of interest since it would appear to indicate little or no increase in glycogen stores at that time of day—7 hours after the morning injection. In previous work with rabbits and cats (8) it was found that sensitivity to glucagon hyperglycemia decreased with repeated doses. However, if these rabbits were maintaining increased glycogen stores, we would expect at least some enhancement of the effect produced by the afternoon injection. That it did not appear may indicate a large fluctuation in liver glycogen stores which should be investigated by killing animals at various times after the glucagon injections.

Whether the glyconeogenesis is a primary effect of glucagon or whether it is secondary to activation of some other endocrine or enzyme system has not been answered by the experiments presented here. However, some possibilities can be ruled out by these results. One mechanism might be an activation of the pituitary-adrenal mechanism by glucagon. However, Helmer and Root (2) found that administration of ACTH to rabbits increased liver glycogen stores in a manner different from that found with glucagon. In ACTH-treated rabbits the total liver glycogen was increased, but the concentration of liver glycogen (mg./gm wet weight of liver) was the same as that of control rabbits. Also, in adrenalectomized rats we found that glucagon increased liver glycogen concentration although not to the levels found in normal animals on the same feeding regimen. These results show that adrenal hormones are not necessary for the production of this effect and, therefore, glucagon cannot be acting indirectly through stimulation of the adrenals or release of ACTH from the anterior pituitary.

The experiment in alloxan diabetic animals indicates that glucagon is probably not causing a stimulation of insulin production since there were few, if any, β-cells remaining in the pancreases of these animals. The glucagon neither improved nor exacerbated the diabetes as judged by urinary glucose excretion; therefore, the mechanism of glyconeogenesis does not seem to involve insulin or any mechanism in which insulin plays a part.

Recently, Sutherland and Wosilait (9) have found that liver phosphorylase can be inhibited by an inactivating enzyme (I.E.) found in the liver and that inactive liver phosphorylase can be reactivated by another enzyme, probably a kinase, also found in liver. Glucagon and epinephrine, in an *in vitro* system, greatly accelerated the activation of liver phosphorylase as measured by the uptake of P^{32} by the enzyme. These authors postulate that glucagon and epinephrine may bring about reactivation of liver phosphorylase through some action on the phosphorylase reactivating system. *In vivo*, the net result of this reaction should be the increase in blood glucose and decrease in liver glycogen which is seen after a single dose of glucagon or epinephrine. It is not possible at present to explain how this action of glucagon can correlate with the great increase in liver glycogen concentration found after repeated injections of glucagon. That glucagon produces any alteration in the phosphorylase system appears unlikely, since each glucagon injection caused hyperglycemia. The increase in liver glycogen concentration may be a rebound type of reaction, but if that is the case one would expect epinephrine to produce a similar increase in liver glycogen concentration rather than the marked decrease which occurred.

It is probable that a more detailed analysis of the time course of the liver glycogen response to glucagon administration would help to clarify this phenomenon.

SUMMARY

Daily injections of large doses of glucagon in rabbits increased liver glycogen stores twofold and increased the blood sugar response to a single dose of glucagon.

In rats, glucagon increased liver glycogen stores by a factor of 10 within three days. Further treatment—up to 14 days—did not greatly increase this response.

Increased liver glycogen stores were produced by daily glucagon injections in alloxan diabetic and adrenalectomized rats.

Daily epinephrine injections decreased liver glycogen stores.

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INHIBITION OF STRESS INDUCED ADRENAL ASCORBIC ACID DEPLETION BY MORPHINE, DIBENZYLINE AND ADRENAL CORTEX EXTRACT

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STIMULATION of the pituitary-adrenal axis by a variety of stressing agents and procedures is a well known phenomenon (1). The mechanism(s) by which different types of stress induce this response remains unknown. The attempt to inhibit the pituitary-adrenal response to stress by various agents represents one approach to this problem. Often such experiments have been exceedingly difficult to interpret because of non-specific stressing effects of the intended blocking agents themselves. In some instances, however, "adapting" procedures (2) or the development of tolerance (3) have been used to circumvent the acute stressing effects.

The present investigation is concerned with attempts to inhibit pituitary-adrenal stimulation in response to various stresses by Dibenzyline,[1] morphine, and adrenal cortex extract. Under the conditions of these experiments these agents did not cause pituitary-adrenal stimulation.

METHODS

White male rats, descendants of the Wistar strain, weighing 125–200 gm., previously anesthetized with sodium pentobarbital (4.0–6.0 mg./100 gm.), were used for all experiments.

The stresses applied were: 1) sham adrenalectomy, 2) unilateral adrenalectomy, 3) l-epinephrine bitartrate injected subcutaneously (doses indicated in tables refer to l-epinephrine base), 4) hydroxyamphetamine HBr[2] injected subcutaneously or intravenously, and 5) vasopressin injected intravenously. In each case, the time from the onset of stress to termination of the experiment was one hour.

In testing for their ability to inhibit pituitary-adrenal responses to the above stresses, adrenal cortex extract (Upjohn) and morphine sulfate were injected subcutaneously; Dibenzyline, in physiological saline solution, was injected intravenously. These agents

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[1] Kindly supplied by the Research and Development Division of Smith, Kline and French Laboratories, Philadelphia 1, Pennsylvania.

[2] Supplied as "Paredrine Hydrobromide" by Smith, Kline and French Laboratories, Philadelphia 1, Pennsylvania.

were administered one hour prior to the onset of stress. Control rats injected by the same route with saline were run simultaneously with each experimental group.

Adrenal ascorbic acid depletion was used as an index of adrenocorticotropin secretion (4). Ascorbic acid was determined by the method of Roe and Kuether (5).

RESULTS

The effects of the various stresses employed on adrenal ascorbic acid concentration are shown in Table 1. While sham adrenalectomy and unilateral adrenalectomy consistently produced significant adrenal ascorbic acid depletion, the effects of epinephrine and hydroxyamphetamine were variable and much less consistent, even with large doses. In addition, in three different experiments (Experiments 1, 2, 3) we were unable to demon-

TABLE 1. STRESS-INDUCED ADRENAL ASCORBIC ACID DEPLETION

Exper. No.	Group	No. of rats	Procedure*	Adrenal ascorbic acid conc. (mg./100 gm.)		P Fisher's table	
				Mean	S. E.**	Compared with group a	Compared with group b
1	a	5	Control-Non-operated	472	25.8		
	b	5	Sham adrenalectomy	342	38.5	<0.05	
	c	5	Unilat. adrenalectomy	270	20.5	<0.01	>0.1
2	a	8	Control	377	20.2		
	b	7	Sham adrenalectomy	288	21.9	<0.02	
	c	7	Unilat. adrenalectomy	275	27.0	<0.01	>0.4
3	a	12	Control	359	11.8		
	b	11	Sham adrenalectomy	301	10.1	<0.01	
	c	13	Unilat. adrenalectomy	282	10.4	<0.01	>0.2
4	a	7	Control-Saline s.c.	414	10.9		
	b	7	l-Epinephrine-0.025 mg.	327	14.2	<0.01	
5	a	8	Control-Saline s.c.	386	23.2		
	b	8	l-Epinephrine-0.037 mg.	318	8.9	<0.02	
6	a	8	Control-Saline s.c.	394	18.5		
	b	8	l-Epinephrine-0.037 mg.	354	9.2	>0.05	
7	a	8	Control-Saline s.c.	347	16.3		
	b	8	l-Epinephrine-0.05 mg.	263	13.9	<0.01	
8	a	8	Control-Saline s.c.	377	8.0		
	b	4	l-Epinephrine-0.1 mg.	256	4.5	<0.01	
	c	5	l-Epinephrine-0.05 mg.	321	8.6	<0.01	
9	a	4	Control-Saline i.v.	358	45.2		
	b	6	Hydroxy-Amphetamine-1.0 mg.	279	20.8	>0.1	
10	a	4	Control-Saline s.c.	452	20.7		
	b	5	Hydroxy-Amphetamine-1.0 mg. s.c.	359	13.2	<0.01	
11	a	9	Control-Saline i.v.	388	12.9		
	b	10	Vasopressin-0.1 μ	240	7.6	<0.01	

* All doses are per 100 gm. body weight.
** Standard error of the mean.

TABLE 2. ADRENAL ASCORBIC ACID CONCENTRATION AFTER ACE, DIBENZYLINE AND MORPHINE

Exper. No.	Group	No. of rats	Procedure*	Adrenal ascorbic acid conc. (mg./100 gm.)		P Fisher's table
				Mean	S. E.	Compared with group a
1	a	9	Control-Saline s.c.	376	28.4	
	b	8	ACE-1 cc.	360	13.9	>0.5
2	a	7	Control-Saline i.v.	367	30.5	
	b	6	Dibenzyline-0.3 mg.	389	23.9	>0.5
3	a	7	Control-Saline i.v.	392	13.6	
	b	8	Dibenzyline-0.3 mg.	404	31.3	>0.5
4	a	13	Control-Saline s.c.	380	9.9	
	b	7	Morphine-2.0 mg.	396	8.4	>0.1

* All doses are per 100 gm. body weight.

TABLE 3. EFFECTS OF ACE, DIBENZYLINE AND MORPHINE ON ADRENAL ASCORBIC ACID DEPLETION PRODUCED BY SHAM ADRENALECTOMY

Exper. No.	Group	No. of rats	Procedure*	Adrenal ascorbic acid conc. (mg./100 gm.)		P (Fisher's table)	
				Mean	S. E.	Compared with group a	Compared with group b
1	a	8	Control-Saline s.c.	412	26.1		
	b	5	Sham Adrenalectomy	274	15.6	<0.01	
	c	6	ACE-1.5 cc. +Sham Adrenalectomy	349	15.9	>0.05	<0.01
2	a	6	Control-Saline s.c.	399	22.7		
	b	6	Sham Adrenalectomy	235	17.3	<0.01	
	c	6	Morphine-2.0 mg. +Sham Adrenalectomy	357	10.3	>0.1	<0.01
3	a	7	Control-Saline s.c.	353	16.6		
	b	7	Sham Adrenalectomy	280	11.9	<0.01	
	c	7	Morphine-2.0 mg. +Sham Adrenalectomy	335	10.7	>0.3	<0.01
4	a	7	Control-Saline s.c.	412	20.8		
	b	8	Sham Adrenalectomy	278	18.6	<0.01	
	c	6	Morphine-2.0 mg. +Sham Adrenalectomy	364	15.3	>0.05	<0.01
5	a	8	Control-Saline s.c.	367	28.4		
	b	7	Sham Adrenalectomy	251	18.4	<0.01	
	c	8	Morphine-2.0 mg. +Sham Adrenalectomy	344	30.4	>0.5	<0.05
6	a	14	Control-Saline i.v.	428	12.9		
	b	7	Sham Adrenalectomy	242	7.9	<0.01	
	c	5	Dibenzyline-0.3 mg.+Sham Adrenalectomy	211	19.8	<0.01	>0.1
7	a	8	Control-Saline i.v.	403	22.1		
	b	7	Sham Adrenalectomy	268	14.2	<0.01	
	c	8	Dibenzyline-0.3 mg.+Sham Adrenalectomy	281	26.5	<0.01	>0.5

* All doses are per 100 gm. body weight.

strate any significant difference between the effects of sham adrenalectomy and unilateral adrenalectomy.

Table 2 shows that under the conditions of these experiments, adrenal cortex extract (ACE), Dibenzyline, or morphine sulfate did not cause adrenal ascorbic acid depletion. These results confirm our previous results for Dibenzyline intravenously (6) and those of Briggs and Munson (3) for morphine sulfate in rats anesthetized with sodium pentobarbital.

Tables 3 and 4 show that ACE and morphine inhibited adrenal ascorbic acid depletion caused by sham adrenalectomy or unilateral adrenalectomy. Morphine sulfate (2.0 mg./100 gm.) appeared to be especially effective; the effects of ACE (1.0 to 2.0 cc./100 gm.) were variable. Dibenzyline was

TABLE 4. EFFECTS OF ACE, DIBENZYLINE AND MORPHINE ON ADRENAL ASCORBIC ACID DEPLETION PRODUCED BY UNILATERAL ADRENALECTOMY

Exper. No.	Group	No. of rats	Procedure*	Adrenal ascorbic acid conc. (mg./100 gm.)		P (Fisher's table)	
				Mean	S. E.	Compared with group a	Compared with group b
1	a	8	Control-Saline s.c.	334	26.8		
	b	7	Unilateral Adrenalectomy	223	13.0	<0.01	
	c	8	ACE-2.0 cc.+Unilateral Adrenalectomy	336	43.1	>0.5	<0.05
2	a	8	Control-Saline s.c.+i.v.	345	18.4		
	b	8	Unilateral Adrenalectomy	199	5.5	<0.01	
	c	8	ACE-1.0 cc.+Unilateral Adrenalectomy	247	18.6	<0.05	<0.05
	d	9	Dibenzyline-0.3 mg.+Unilateral Adrenalectomy	204	14.5	<0.01	>0.5
	e	8	Morphine-2.0 mg.+Unilateral Adrenalectomy	339	19.3	>0.5	<0.01
3	a	8	Control-Saline s.c.+i.v.	337	30.2		
	b	9	Unilateral Adrenalectomy	180	11.9	<0.01	
	c	11	ACE-1.5 cc.+Unilateral Adrenalectomy	267	18.0	<0.05	<0.01
	d	9	Dibenzyline-0.3 mg.+Unilateral Adrenalectomy	170	10.2	<0.01	>0.4
	e	8	Morphine-2.0 mg.+Unilateral Adrenalectomy	308	10.0	>0.5	<0.01
4	a	8	Control-Saline s.c.	348	15.8		
	b	8	Unilateral Adrenalectomy	272	14.9	<0.01	
	c	6	Morphine-2.0 mg.+Unilateral Adrenalectomy	368	23.7	>0.4	<0.01
5	a	5	Control-Saline i.v.	370	12.6		
	b	6	Unilateral Adrenalectomy	263	13.3	<0.01	
	c	6	Dibenzyline-0.3 mg.+Unilateral Adrenalectomy	285	9.2	<0.01	>0.1
6	a	8	Control-Saline i.v.	393	20.6		
	b	6	Unilateral Adrenalectomy	277	12.9	<0.01	
	c	6	Dibenzyline-0.3 mg.+Unilateral Adrenalectomy	272	17.8	<0.01	>0.5

* All doses are per 100 gm. body weight.

consistently ineffective in inhibiting adrenal ascorbic acid depletion induced by these stresses.

Tables 5 and 6 show that the adrenal ascorbic acid depletion caused by epinephrine or hydroxyamphetamine can be inhibited by morphine or ACE and, in addition, by Dibenzyline. This finding confirms our previous results with Dibenzyline (6). In these experiments Dibenzyline also blocked other effects (piloerection, salivation, lacrymation, urination, exophthalmos

TABLE 5. EFFECTS OF ACE, DIBENZYLINE AND MORPHINE ON ADRENAL ASCORBIC ACID DEPLETION PRODUCED BY l-EPINEPHRINE

Exper. No.	Group	No. of rats	Procedure*	Adrenal ascorbic acid conc. (mg./100 gm.)		P (Fisher's table)	
				Mean	S. E.	Compared with group a	Compared with group b
1	a	9	Control-Saline sc. +i.v.	448	35.3		
	b	9	l-Epinephrine-0.05 mg.	233	9.9	<0.01	
	c	9	ACE-1.0 cc. +l-Epinephrine-0.05 mg.	361	27.9	>0.05	<0.01
	d	9	Dibenzyline-0.3 mg. +l-Epinephrine 0.05 mg.	288	17.0	<0.01	<0.02
	e	8	Morphine-2.0 mg. +l-Epinephrine-0.05 mg.	350	12.8	<0.05	<0.01
2	a	7	Control-Saline i.v.	381	21.1		
	b	8	l-Epinephrine-0.06 mg.	278	17.0	<0.01	
	c	8	Dibenzyline-0.3 mg. +1-Epinephrine-0.06 mg.	360	18.5	>0.4	<0.01
3	a	8	Control-Saline s.c.	386	23.2		
	b	8	l-Epinephrine-0.037 mg.	318	8.9	<0.02	
	c	8	Morphine-2.0 mg. +l-Epinephrine-0.037 mg.	373	15.3	>0.5	<0.01

* All doses are per 100 gm. body weight.

and blanching of the ears) seen following injection of these amines in rats. In Experiment 1, the blocking effect of Dibenzyline was borderline.

Table 7 shows that adrenal ascorbic acid depletion induced by vasopressin can be inhibited by morphine sulfate in sufficient dosage, confirming the results of Briggs and Munson (3). Dibenzyline was completely ineffective in inhibiting the response to this stress. Even with large doses, ACE appeared to have very little effect against vasopressin induced adrenal ascorbic acid depletion.

DISCUSSION

The results of this investigation indicate that stress-induced adrenal ascorbic acid depletion may be inhibited by appropriate pharmacologic agents. Further, the inhibition of adrenal ascorbic acid depletion obtained by each of these agents varies with the type of stress applied. Dibenzyline blocked only the responses to epinephrine and hydroxyamphetamine; the responses to operative stress and to vasopressin were unaffected. Large

TABLE 6. EFFECTS OF ACE, DIBENZYLINE AND MORPHINE ON ADRENAL ASCORBIC ACID DEPLETION PRODUCED BY HYDROXY-AMPHETAMINE

Exper. No.	Group	No. of rats	Procedure*	Adrenal ascorbic acid conc. (mg./100 gm.)		P (Fisher's table)	
				Mean	S. E.	Compared with group a	Compared with group b
1	a	8	Control-Saline s.c.	373	22.1		
	b	8	Hydroxy-Amphetamine-1.5 mg. s.c.	299	12.5	<0.02	
	c	8	ACE-1.0 cc. + Hydroxy-Amphetamine-1.5 mg. s.c.	352	12.1	>0.4	<0.01
2	a	6	Control-Saline s.c.	382	10.9		
	b	7	Hydroxy-Amphetamine-1.5 mg. s.c.	316	24.5	<0.05	
	c	6	Dibenzyline-0.3 mg. + Hydroxy-Amphetamine-1.5 mg. s.c.	405	10.7	>0.1	<0.05
3	a	10	Control-Saline s.c. + i.v.	388	13.3		
	b	11	Hydroxy-Amphetamine-2.0 mg. s.c.	304	20.8	<0.01	
	c	10	Morphine-0.5 mg. + Hydroxy-Amphetamine-2.0 mg. s.c.	420	16.7	>0.05	<0.01

* All doses are per 100 gm. body weight.

TABLE 7. EFFECTS OF ACE, DIBENZYLINE AND MORPHINE ON ADRENAL ASCORBIC ACID DEPLETION PRODUCED BY VASOPRESSIN

Exper. No.	Group	No. of rats	Procedure*	Adrenal ascorbic acid conc. (mg./100 gm.)		P (Fisher's table)	
				Mean	S. E.	Compared with group a	Compared with group b
1	a	11	Control-Saline i.v.	372	15.5		
	b	13	Vasopressin-0.07 μ.	319	11.0	<0.02	
	c	11	ACE-2.0 cc. + Vasopressin-0.07 μ.	347	14.1	>0.2	>0.1
	d	11	Morphine-1.0 mg. + Vasopressin-0.07 μ.	358	10.0	>0.4	<0.05
2	a	9	Control-Saline i.v. + s.c.	388	12.9		
	b	10	Vasopressin-0.1 μ.	240	7.6	<0.01	
	c	8	ACE-2.0 cc. + Vasopressin-0.1 μ.	286	14.9	<0.01	<0.01
	d	12	Morphine-0.5 mg. + Vasopressin-0.1 μ.	300	11.2	<0.01	<0.01
3	a	11	Control-Saline i.v. + s.c.	437	11.8		
	b	11	Vasopressin-0.1 μ.	340	12.5	<0.01	
	c	13	ACE-2.5 cc. + Vasopressin-0.1 μ.	341	13.4	<0.01	>0.5
	d	13	Morphine-1.0 mg. + Vasopressin-0.1μ.	400	17.6	>0.1	<0.02
4	a	7	Control-Saline i.v. + s.c.	432	9.6		
	b	6	Vasopressin-0.1 μ.	336	22.7	<0.01	
	c	6	Dibenzyline-0.3 mg. + Vasopressin-0.1 μ.	330	13.3	<0.01	>0.5

* All doses are per 100 gm. body weight.

doses of ACE blocked the responses to the sympathomimetic amines and to operative stress, but had little or no effect on the response to vasopressin. Morphine, in large doses, was the only agent which inhibited the responses to all of the different stresses applied.

The failure of Dibenzyline, in adequate blocking doses, to inhibit the adrenal ascorbic acid depletion caused by operative stress and by vasopressin strongly suggests that epinephrine is not involved in the pituitary-adrenal responses to these stresses. Guillemin (2) has also presented evidence that epinephrine is not involved in the response to the stress of formalin or forced immobilization. That epinephrine plays any fundamental role in the pituitary-adrenal response to stress appears to us as a very unlikely possibility. The adrenal ascorbic acid depletion induced by epinephrine is highly variable, even when large doses are used. In addition, Sandberg, Nelson, Palmer, Samuels and Tyler (7) were unable to demonstrate any increase in blood or urinary 17-hydroxycorticosteroids following intravenous or subcutaneous administration of epinephrine to normal humans. Indeed, very small doses of epinephrine have been reported (8) to reduce plasma ACTH in adrenalectomized rats, and Sandberg and associates (7) observed that the increase in plasma 17-hydroxycorticosteroids following concomitant ACTH and epinephrine administration was less than the rise following ACTH alone.

Numerous studies have indicated that pre-treatment with adrenal cortical hormones will inhibit the pituitary-adrenal response to a variety of stressful stimuli (1). The amount of cortical hormone necessary for this inhibition is greater with increasing intensity of the stress (9). In addition, ACTH is present in greater concentration in the blood of patients with Addison's disease than in the blood of normal humans (10, 11). Thus, there is considerable evidence in favor of the concept of pituitary-adrenal regulation proposed by Sayers and Sayers (1, 9), according to which ACTH secretion is regulated by a feedback mechanism. Our results indicate that ACE in doses up to 2.5 cc. per 100 gm. can inhibit adrenal ascorbic acid depletion in rats in response to operative stress or to sympathomimetic amines. On the other hand, ACE exhibited at best only a very questionable inhibitory effect vasopressin-induced adrenal ascorbic acid depletion, and, judging from the degree of depletion, the doses of vasopressin used did not appear to give maximal stimulation. In addition, in three separate experiments, we were unable to demonstrate that unilateral adrenalectomy is quantitatively any more effective as a stress than sham adrenalectomy.

Our results confirm those of Briggs and Munson (3) that morphine is capable of blocking the adrenal ascorbic acid depletion in response to a variety of stimuli. Morphine does not prevent the action of injected ACTH on adrenal ascorbic acid (3). Morphine is capable of blocking the response to all of the stresses applied by us (sham adrenalectomy, unilateral adrenalectomy, epinephrine, hydroxyamphetamine, and vasopressin) and to all those applied by Briggs and Munson (histamine, epinephrine, vasopressin,

laparotomy and unilateral adrenalectomy) (3). In unanesthetized rats, morphine itself will cause adrenal ascorbic acid depletion (3, 12) which is dependent on an intact pituitary (12). As our results indicate and as shown previously by Briggs and Munson (3), morphine does not cause adrenal ascorbic acid depletion following sodium pentobarbital anesthesia. On the assumption that morphine acts on the central nervous system and not directly on the anterior pituitary, Briggs and Munson (3) interpret their results as evidence in favor of a central neural or neurohumoral mechanism for the regulation of ACTH secretion. The evidence for a central neural or neurohumoral mechanism regulating ACTH secretion has recently been summarized by Harris and Fortier (13).

Of the various hypotheses proposed for the regulation of ACTH secretion, each leaves much to be desired. Our results, along with those of other investigators, suggest that several steps may be involved in the pathway for the stimulation of ACTH secretion, and that the sites of some of these steps are susceptible to blockade by different agents or procedures. Pentobarbital anesthesia will inhibit the response to mild sensory stimulation, cold, and morphine, but not to a variety of other stresses (1, 3). Hypothalamic lesions (13) inhibit the responses to a variety of stimuli such as operative stress, sound and immobilization; the effects on the responses to epinephrine, histamine, and cold are contradictory. According to McCann and Brobeck (14) hypothalamic lesions which prevent the response to unilateral adrenalectomy, epinephrine and histamine do not prevent the response to large doses of vasopressin. ACE, in large doses, also inhibits the responses to a wide variety of stimuli (operative stress, cold, formalin, epinephrine, histamine, etc.) but not to vasopressin (even with doses of vasopressin causing less than maximal stimulation). Morphine, on the other hand, has inhibited the responses to all types of stress so far tested, which include vasopressin, unilateral adrenalectomy, sham adrenalectomy, laparotomy, histamine, epinephrine and hydroxyamphetamine.

These data suggest to us that different stresses may stimulate at different sites along the pathway for the stimulation of ACTH secretion; and that hypothetical sites A, B, and C, progressing toward the anterior pituitary, may be blocked by pentobarbital, by ACE or hypothalamic ablations, and by morphine respectively. Stimuli such as acetylcholine, histamine, and epinephrine appear to be specifically blocked by the respective blocking agents (2, 6) at their sites of action and also by non-specific blockade at or subsequent to the site at which they stimulate. Except in the case of hypothalamic lesions the exact *site* at which stimulation or blockade occurs remains unknown. Finally, the fundamental *mechanisms* by which blockade or stimulation is obtained is completely unknown. Perhaps the application of more direct methods for measuring ACTH and adrenal cortical hormone secretion will help resolve some of the difficulties.

SUMMARY

Adrenal ascorbic acid depletion induced by a variety of stresses (sham adrenalectomy, unilateral adrenalectomy, l-epinephrine, hydroxyamphetamine, vasopressin) can be inhibited by appropriate pharmacologic agents. In this investigation, the agents tested for their ability to inhibit the pituitary-adrenal response to stress were: adrenal cortex extract (ACE), morphine, and Dibenzyline; under the conditions of these experiments these agents did not by themselves produce adrenal ascorbic acid depletion. Morphine was the only agent which inhibited the responses to all the stresses applied. ACE blocked the response to operative stress and to the sympathomimetic amines, but not the response to vasopressin. Dibenzyline blocked only the responses to the sympathomimetic amines. These findings are discussed in relation to the regulation of pituitary-adrenal function.

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A COMPARISON OF TWO GROWTH HORMONE PREPARATIONS

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IN EXPERIMENTS conducted in our laboratories (G. H. Beaton *et al.* (1, 2, 3), we have used two different sources of anterior pituitary growth hormone. In the earlier studies Connaught growth hormone was employed, but more recently we have used an Armour preparation. In view of the increasing interest in the source of growth hormone preparations and the possibility that the results obtained from different preparations would not be comparable, it was decided to test the effects of these two hormones in adult female rats. Since the objective of this study is to check possible differences, we have selected criteria which have been found to change after hormone treatment in previous experiments. The data may be useful to other workers in this field.

METHODS AND MATERIALS

Adult female rats of the Wistar strain from the Carworth Farms colony were employed in this study. Forty rats weighing between 200 and 240 grams were maintained for 10 days on a 20% casein 20% corn oil diet. This diet was prepared as has been described previously (J. R. Beaton *et al.* 4) except that a nonnutritive cellulose bulk was added in place of agar and vitamin B_6 was added at a level of 5 mg. per 1000 gm. of diet. At the end of the 10-day equilibration period, the animals were weighed and divided into 4 groups of ten rats each; each group had an initial average body weight of 217 gm. One group was fasted for 18 hours and then killed as an initial control. The remaining three groups were maintained on the complete diet fed *ad libitum*. One of these groups was given ½ ml. of saline per rat per day; another group was given 3.0 mg. per rat per day of Connaught growth hormone[1] in ½ ml. saline; the last group was given 3.0 mg. per rat per day of Armour growth hormone[2] in ½ ml. saline. All injections were subcutaneous. In all, 14 daily injections were given to each rat, and then the animals were fasted for 18 hours and killed by stunning and decapitation. Blood was collected from the neck in heparinized tubes. Livers were removed immediately, blotted, weighed, and then samples taken for the determination of enzyme activities. The carcasses were pooled by groups and quickly frozen; at a later date, they were passed through a power grinder and samples taken for the determination of carcass composition. Livers were also pooled by groups and frozen; later they were homogenized in a Waring blendor and samples taken for the determination of their composition.

Blood and enzyme analyses were carried out by the following procedures:—blood urea, Archibald (5); blood amino nitrogen, Frame *et al.* (6); packed cell volume by the standard procedure; aspartic-glutamic transaminase activity by the method of Tonhazy *et al.* (7)

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[1] Anterior pituitary growth hormone, Connaught Laboratories, Lot #100–1.

[2] Somar, Armour Laboratories, Lot #208.

and alanine-glutamic transaminase activity by this method as modified by Caldwell and McHenry (8); liver arginase activity by the method of Van Slyke and Archibald (9) as adapted to liver tissue by Liener and Schultze (10). Analyses were carried out on the pooled carcass and liver samples by the following procedures:—nitrogen by the standard macro-Kjeldahl procedure, multiplying by 6.25 to give protein; moisture by the standard procedure of drying in aluminum dishes (A.O.A.C.) at 105° C for 5 hours; total crude fatty acids by the method of Gavin and McHenry (11). Statistical analyses were carried out by means of the "t"-test.

RESULTS AND DISCUSSIONS

During the experimental period both growth hormone preparations caused identical average body weight gains, as follows:—saline, 16±9 gm./rat; Armour, 38±7 gm./rat; Connaught, 38±3 gm./rat. The gains of the

TABLE 1. EFFECTS OF TWO GROWTH HORMONE PREPARATIONS ON BODY COMPOSITION

Group	% composition			Net change from initial (gm.)		
	Fat	Protein	Moisture	Fat	Protein	Moisture
			A. Carcass			
Initial	17.6	19.8	58.3	—	—	—
Saline	24.2	19.2	53.2	+16.2	+1.5	− 3
Connaught G. H.	18.3	19.4	59.0	+ 7.1	+7.1	+26
Armour G. H.	16.5	19.6	59.9	+ 3.2	+7.2	+27
			B. Liver			
Initial	5.4	23.5	69.3	—	—	—
Saline	4.5	24.3	69.7	− 0.1	0	−0.1
Connaught G.H.	4.4	22.6	70.6	0	+0.2	+1.0
Armour G.H.	4.3	22.3	70.9	0	+0.3	+1.2

hormone-treated rats were significantly greater than that of the control group. As has been observed in previous experiments (Beaton and Curry, 3) there was very little change in food intake during treatment with growth hormone. The average food intakes, expressed in gm./rat/day, were as follows:—saline 12.0, Connaught growth hormone 14.8, Armour growth hormone 13.1. The composition of the body weight gain can be seen in Table 1. It is apparent that the two growth hormone preparations produced analogous effects. In both cases there was a slight increase in the protein content of the livers and a marked increase in body protein. In both cases, also, there was a reduced retention of fat in the carcasses, an observation which is in accord with previous results (3). However, the Connaught preparation induced an increase in food intake not evident in the animals given the Armour preparation. The increased food intake in the Connaught rats partially nullified the effect of growth hormone on fat retention.

The biochemical alterations shown in Table 2 illustrate the close similarity of the effects of the two hormones. The only marked discrepancy is that the Connaught hormone significantly reduced the liver arginase activity whereas the Armour preparation had no effect on this enzyme. Both hor-

mones caused a depression in the blood urea; however this was only significant among the rats treated with the Connaught hormone. Other studies have revealed a significant lowering of this metabolite with either hormone. It seems likely therefore that although the Armour preparation may not affect liver arginase activity, it does affect urea metabolism. With regard to the other alterations seen, it is noted that there are three quantitative differences. Connaught growth hormone produced a significantly (1%) greater depression of alanine-glutamic transaminase activity than did the Armour preparation; the aspartic-glutamic enzyme was affected by both hormones to the same extent. On the other hand, the Armour preparation caused a greater increase in liver weight than did the Connaught hormone

TABLE 2. BIOCHEMICAL ALTERATIONS CAUSED BY GROWTH HORMONE TREATMENT (mean ±S.D.)

Assay	Initial	Saline	Connaught G.H.	Armour G.H.
Liver Assays				
Aspartic-glutamic Transaminase[1]	142 ±11	144 ±12	121 ±8[3]	124 ±12[3]
Alanine-glutamic Transaminase[1]	63 ±14	58 ±15	31 ±5[3]	40 ±8[3]
Arginase[2]	33 ±6	45 ±8	35 ±3[3]	44 ±7
Liver Wt. (% Body Wt.)	2.71 ±0.14	2.45 ±0.16	2.73 ±0.17[3]	2.91 ±0.16[3]
Blood Assays				
Amino nitrogen	9.3 ±0.3	10.4 ±1.0	12.3 ±0.6[3]	12.3 ±0.4[3]
Urea	23.1 ±5.3	28.4 ±4.7	22.5 ±4.6[4]	25.0 ±7.2
Packed Cell Volume	46.5 ±1.5	46.7 ±1.4	43.8 ±1.5[3]	45.6 ±1.2

[1] Expressed as μl pyruvate-CO_2 formed per mg. wet tissue per hour.
[2] Expressed as μmol urea formed per 100 mg. wet tissue per hour.
[3] Significantly different from the mean for the saline group at the 1% level.
[4] Significantly different from the mean for the saline group at the 5% level.

(significant at the 5% level). A difference was also seen with respect to packed cell volume which was significantly lowered by the Connaught preparation but only slightly lowered by the Armour hormone.

It would appear that certain differences do exist in the activities of these two hormones, especially with respect to liver arginase activity. However, in general, the two hormones produce quite similar changes. The differences may be due to relative potencies or to contamination with other pituitary hormones. The dosages were in terms of the absolute weight of the two hormones, and not in terms of standard preparations. However, the dosages could not have been greatly different in view of the identical body weight gains. Unfortunately, accurate information as to the exact contaminants in these preparations is not available. Nevertheless, the results do indicate that results obtained with these two hormones are comparable and that the two hormone preparations may be used interchangeably in metabolic studies in rats.

SUMMARY

The effects of the administration of Armour and Connaught growth hormones have been compared in adult female rats. While some differences

in response between the two hormones have been noted, the effects are comparable.

Acknowledgments

Armour growth hormone was supplied, free of charge, through the courtesy of Dr. Irby Bunding. Connaught growth hormone was made available through a grant from the National Research Council of the Government of Canada. This work was greatly facilitated by a Public Health Research Grant from the Department of National Health and Welfare of the Government of Canada.

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ALDOSTERONE AND HYDROCORTISONE SECRETION BY THE DENERVATED ADRENAL[1]

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IN THE course of a systematic investigation of the physiological factors which influence the adrenal output of aldosterone, the effect of denervation of the adrenal on the secretion of this potent electrolyte-active steroid was determined. That the sympathetic nerve supply to the gland might influence corticosteroid output has been suggested by several workers. Vogt (1) described an increase in cortical hormone in the adrenal vein following splanchnic stimulation. Okinaka and collaborators (2) claim to have induced a fourfold increase in corticosteroid output by splanchnic stimulation. More recently, Rosenfeld (3) has found the output of 17-hydroxylated corticoids by isolated perfused calf adrenals to be increased by the addition of acetylcholine to the perfusion mixture. Rosenfeld has suggested that the liberation of acetylcholine at preganglionic endings of the sympathetic nerves to the gland represents a mechanism for neural control of the adrenal cortex.

It is of interest also to compare the aldosterone output of the isolated perfused bovine adrenal with that of the dog adrenal *in situ*. Ungar, Rosenfeld, Rosemberg and Dorfman (4, 5) have reported the aldosterone secretion rate of isolated perfused glands to be of the order of 0.3 μg./gram adrenal per hour. This is only about 10% of the rate of secretion of aldosterone by the dog adrenal *in situ* (2.98 μg./gram of adrenal/hour, on the average (6)). Neglecting the experimental variables associated with nonspecific trauma incident to perfusion, this discrepancy might be ascribed to (1) the absence in the perfusion mixture of metabolic precursors or of a specific aldosterone-stimulating factor, or (2) to the absence of the sympathetic outflow to the adrenal. The question could be resolved by determining the secretion rates of the steroid in transplanted adrenals in which all nerve supply was interrupted.

To this end adrenals were transplanted from donor dogs to the thighs of recipient dogs in acute experiments. The results indicate that aldosterone output in transplanted glands is at least as high as in intact glands *in situ*.

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[2] Experimental work was conducted in partial fulfillment of the Research Project Teaching Program at Western Reserve University School of Medicine.

The rate of secretion of hydrocortisone was also unchanged from the control rate in intact glands. This finding indicates that the high rate of steroid output by the adrenal *in situ* as compared to the isolated gland is related to a substance or substances present in the blood supply and not to the sympathetic outflow.

METHODS

Six mongrel dogs were used. Three dogs served as donors and three as recipients of the transplanted adrenals. Each donor animal was anesthetized with sodium pentobarbital and a lateral incision was made between the iliac crest and the 12th rib. The left lumbo-adrenal vein was cannulated. Sufficient heparin was infused to prevent clotting. The renal, lumbar, coeliac and superior mesenteric arteries were ligated and sectioned. The aorta was ligated and sectioned proximal to the coeliac and distal to the renal arteries. The left adrenal gland with its vascular connections intact and the segment of aorta between the coeliac and renal arteries were removed and introduced into a subcutaneous pocket in the medial aspect of the thigh of the recipient dog previously anesthetized and prepared. Vascular connections were made between the aortic segment of the transplanted gland and the left femoral artery of the recipient dog. The lapse of time between removal of the gland from the donor dog and completion of the vascular connections to the recipient dog was on the average 20 minutes. Adrenal venous flow began immediately on completion of the transplant and was continued for 3–4 hours. The blood flow through the transplanted gland was on the average 1.7 cc. per minute, which compares favorably with that in intact glands (2–5 cc. per minute). Blood volume of the recipient dog was maintained by the infusion of whole dog blood at approximately the same rate as the blood flow from the transplanted gland.

The adrenal venous blood from the transplanted gland was collected in vessels kept at 0° C. The blood was diluted 1:1 with water and extracted twice with a volume of chloroform equal to the blood-water mixture. Aldosterone and hydrocortisone were isolated by paper chromatographic methods, the details of which have been published (7). Evidence for the identity of aldosterone is as follows. An aliquot of the isolated material combined with an equal amount of authentic aldosterone diacetate chromatographed as a single component. The sulfuric acid chromogen was identical with that of a sample of authentic aldosterone examined under the same conditions. An aliquot of the isolated steroid was subjected to hydrolysis (acetyl-cholinesterase, glycyl-glycine buffer pH 7.4, 4 hours, 30° C) and rechromatographed 48 hours on paper in the system propylene glycol-toluene. A fraction separated which migrated at the rate of free aldosterone. This was assayed for sodium retaining activity in adrenalectomized rats at doses of 0.113 and 0.452 μg. It was found to have a potency of 46 ± 13.8 times that of desoxycorticosterone acetate, which is consistent with the known biological activity of aldosterone. The aldosterone was quantitated by absorption at 240 mμ, $\epsilon = 15{,}850$.

RESULTS

The secretion rates of 17-hydroxycorticosterone by the transplanted adrenal glands were 25.4, 26.7 and 23.0 μg./Kg./hour, respectively. The average secretion rate (25.0 μg./Kg./hour) is not significantly different from that of the gland *in situ* (7, 8). The rates of secretion of aldosterone by the transplanted adrenals were 31.7, 52.6 and 55.9 μg./100Kg./hour, respectively. The average of the three experiments was 46.7 μg./100 Kg./hour, which can be compared with the average rate of aldosterone secretion

(21.8±2.99) obtained in 19 intact dogs studied in this laboratory concurrently with this experiment. It was of considerable interest to note that the aldosterone output by the transplanted glands actually appeared to be higher than that in the intact controls. Although the small number of cases studied precludes a final statement, the probability of obtaining the higher value (46.7 μg./100 kg. body weight/hour) in the transplanted glands by random selection from the control population is quite small ($P < .03$).

Expressed as rate of secretion per gram of adrenal tissue, the aldosterone output was on the average 8.3 μg. per gram adrenal per hour. This can be compared with an average of 2.98 μg. per gram per hour obtained in intact dogs (6), and again suggests an increase over the control level. When one compares the rate of secretion of the transplanted dog adrenals with that reported for the isolated perfused calf adrenals (4, 5), the discrepancy between these preparations is quite striking. The rate of aldosterone secretion by the perfused calf adrenals (0.3 μg./gram adrenal tissue per hour) is less than 4% that of the transplanted dog glands. This observation raises serious question as to the physiological significance of studies carried out on the perfused bovine glands.

Rauschkolb and Farrell (9) have recently obtained evidence that aldosterone secretion is controlled by a circulating tropic hormone elaborated by the diencephalon. The findings reported here are in accord with this concept inasmuch as the stimulus to adrenal aldosterone output appears to be a blood-borne agent. These experiments are of interest also in relation to a possible homeostatic regulation of aldosterone secretion. It should be recalled that the adrenals of the *recipient* dogs in this experiment were intact. This may be compared with the situation in the 19 control experiments (6) in which the adrenal effluent of one gland was removed for analysis, thus depriving the animal of the secretory products of that gland. Sydnor (10) has shown that unilateral adrenalectomy in the dog induces an increased level of circulating ACTH, in accordance with the concept of homeostatic regulation of pituitary-adrenal activity elucidated by Sayers (11). It might be postulated that the same reciprocal relationship exists between the release of an aldosterone-stimulating factor and circulating levels of aldosterone. If this were the case, aldosterone output by the adrenal transplanted into the thigh of a recipient animal receiving a normal endogenous supply of aldosterone from two intact adrenal glands would be expected to be lower than in the adrenal of a control animal deprived of the aldosterone output of the cannulated gland. This was clearly not the case, for the aldosterone output appeared to be higher in the transplanted adrenals than in the controls. Although a reciprocal relationship may exist between adrenal aldosterone output and the release of an aldosterone-stimulating tropic hormone, these findings suggest that it is not the only or the dominant mechanism for regulation of aldosterone secretion.

SUMMARY

In order to elucidate the effects of denervation on the secretion of hydrocortisone and aldosterone by the adrenal of the dog, the adrenal gland was transplanted into the thigh in three experiments. Aldosterone and hydrocortisone were isolated from the adrenal effluent by paper chromatographic methods. The rate of secretion of hydrocortisone by the transplanted glands was not significantly different from that of glands *in situ*. The rate of secretion of aldosterone was as high or higher than that in intact glands. It is concluded that the presence of the nerve supply to the adrenal probably plays no major role in the adrenal secretion of either steroid. The data are consistent with the view that the secretion of aldosterone is under the control of a circulating hormone.

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ADRENAL HORMONES AND PHOSPHOLIPID METABOLISM IN LIVER CYTOPLASM OF ADRENALECTOMIZED MICE[1]

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A PHYSIOLOGIC 24-hour periodicity has earlier been noted in some aspects of phosphorus metabolism in the liver (1). During a period of low blood eosinophil counts, the relative specific activity of hepatic phospholipid phosphorus was higher than during a period of high eosinophil counts. These observations were made on independent groups of intact or sham-operated mice, sacrificed by day or by night, one or two hours following the injection of radiophosphorus (P^{32}) (1, 2). Adrenalectomized mice, studied concomitantly, showed, by contrast, a significant inhibition of the day-night differences in the relative specific activity of phospholipid phosphorus from liver cytoplasm. In the course of this work, it also was noted that this relative specific activity was lower in adrenalectomized mice than in sham-operated mice of the same genetic background, sex, and age, irrespective of the time of day of sampling. The effect of certain adrenal hormones upon the lowered relative specific activity of phospholipid phosphorus will be reported herein.

MATERIALS AND METHODS

Several groups of male CBC mice, 6–8 weeks of age were adrenalectomized under nembutal anesthesia. For at least 7 days prior to the operation, the mice were kept singly housed, in a room maintained at $78 \pm 1°$ F, illuminated from 06:00 to 18:00 and kept dark from 18:00 to 06:00. Purina Fox Chow and tap water were available to the mice *ad libitum*, from the time of weaning, throughout the post-operative period, until two hours before sacrifice. Salt was not added to the drinking water of the adrenalectomized mice. These mice were all sacrificed on the third day after adrenalectomy, at 23:00. Four hours before sacrifice, different groups of mice received intraperitoneal injections of 0.9% sodium chloride, epinephrine hydrochloride, adrenocorticotropin (ACTH, 1 unit/mg.), cortisone acetate,[2] or intramuscular injections of desoxycorticosterone[3] acetate. Each mouse was weighed before injection and the doses given were adjusted

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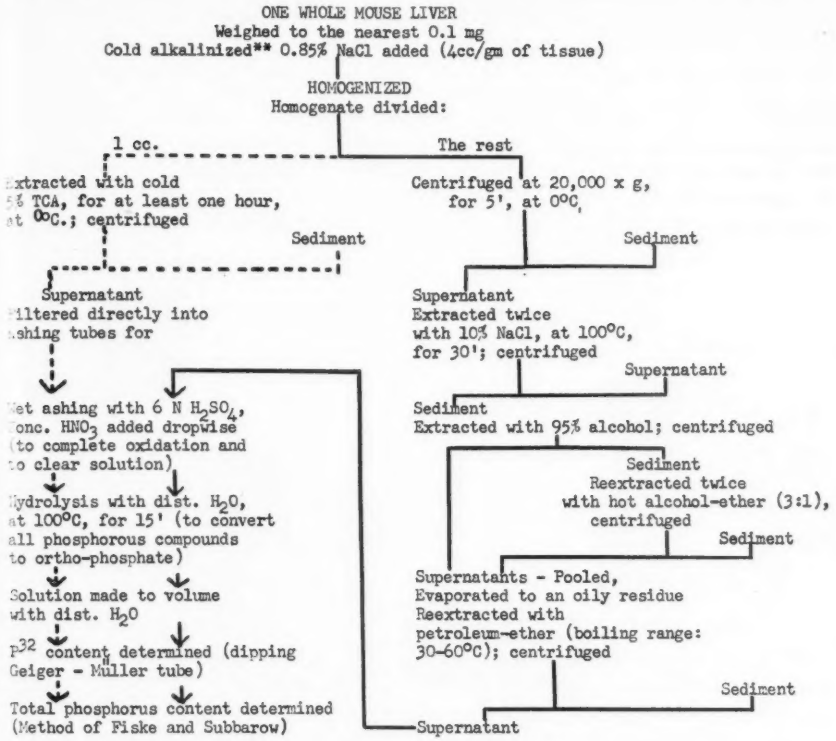

* Relative specific activity = $\frac{\text{counts/minute/gamma of phospholipid phosphorus}}{\text{counts/minute/gamma of acid-soluble phosphorus}} \times 100$

** 2 cc of 0.1 N NaOH/liter of saline

FIG. 1. Determination of relative specific activity* of phospholipid phosphorus in a cytoplasmic fraction of mouse liver.

to refer to 20 gm. of body weight and 0.2 cc. of volume. The several treatments were given in rotation, in order to minimize the possible role played by a time factor difference among treatments.

In each of the experiments, P^{32} was injected intraperitoneally, exactly two hours prior to sacrifice. P^{32} was given as $H_3P^{32}O_4$, in a solution containing 100 microcuries per ml., each mouse receiving 1 microcurie per gm. of body weight. For the two-hour period between injection and sacrifice, the mice were kept in mason jars, without food or water. Immediately following sacrifice, the livers were removed and processed as outlined in Figure 1. Solutions designated as "cold" in this scheme were kept in an ice bath

[2] Kindly provided by Merck and Co., Rahway, New Jersey, through the courtesies of Dr. E. Alpert and Dr. L. Sarett.

[3] Kindly provided by Ciba and Co., Summit, New Jersey, through the courtesy of Dr. E. Oppenheimer.

prior to and during their use. Potter-Elvehjem glass homogenizers were employed, and were also kept in the ice bath. Ashing was carried out on a hot sandbath. Radioactivity of samples was determined by the use of a TGC 5 Tracerlab dipping counter attached to a regular commercial amplification unit. A minimum of 10,000 counts per sample was obtained, and the computed counts per minute were then corrected for counter loss, background and time-decay. In order to compute the relative activity of the phospholipid phosphorus, the corrected counts per minute per μg. of phosphorus of the phospholipid samples were related to the corresponding value for the acid soluble phosphorus oxidized and hydrolyzed in the same way (see scheme in Figure 1).

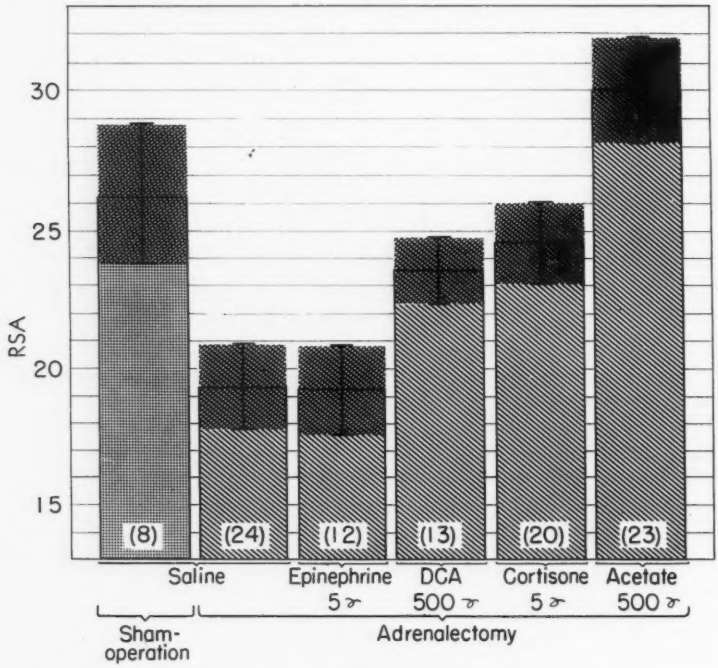

FIG. 2. Effect of certain hormones upon the lowered relative specific activity of phospholipid phosphorus from liver cytoplasm of adrenalectomized male CBC-mice. P^{32} injected 2 hours prior to sacrifice. Numbers in parentheses indicate number of mice in a given group. Data on ACTH group are not graphed (see Table 1). RSA = Relative specific activity (see footnote to Fig. 1).

RESULTS

Figure 2 summarizes values for the relative specific activity obtained in the course of this study on adrenalectomized mice given certain hormone treatments or saline. While comparable data obtained earlier (3) on sham-operated mice given saline are included as a first column in Figure 2, the analysis of treatment effects, presented in Table 1, is based solely upon the data on adrenalectomized mice. The effect of cortisone, in raising the relative specific activity of phospholipid phosphorus from liver cytoplasm of

adrenalectomized mice, stands out clearly in Table 1 as well as in Figure 2. The effect is readily apparent at the 5 μg. dose level; it is more marked for the 500 μg. dose and it is statistically significant at both dose levels. By comparison, the effect of 500 μg. of desoxycorticosterone is relatively small: it is only of borderline significance, and, on a μg. per μg. basis, it compares unfavorably with the effect of cortisone. Figure 2 and Table 1 further reveal that epinephrine or ACTH did not affect the relative specific activity of phospholipid phosphorus from liver cytoplasm of adrenalectomized mice, in the doses and under the circumstances of this study.

TABLE 1. SIGNIFICANCE OF EFFECTS INVESTIGATED

Treatment	Dose (γ)	D.F.	Difference ±SE*	t	P
Cortisone	5	42	+5.24 ±2.18	2.40	.0164
Cortisone	500	45	+10.57 ±2.36	4.48	<.0001
Desoxycorticosterone	500	35	+4.13 ±2.26	1.83	.0672
Epinephrine	5	35	−0.06 ±2.43	0.02	.9840
ACTH	400	34	+1.77 ±2.57	0.69	.4902

* Relative specific activity of phospholipid phosphorus in liver cytoplasm of adrenalectomized mice given the treatment indicated four hours prior to sacrifice *minus* the corresponding value for adrenalectomized mice given 0.9% saline solution. P^{32} injected two hours before sacrifice (see text).

DISCUSSION

Cortisone raises the relative specific activity of phospholipid phosphorus from liver cytoplasm of adrenalectomized mice from levels which are clearly below the physiologic range to values which appear to be within that range. More important, cortisone does so in doses which may be regarded as physiologic (5 μg./20 gm. of body weight), while the same doses of epinephrine do not exhibit the effect. Furthermore, desoxycorticosterone, in doses one hundredfold larger, barely reproduces the effect of 5 μg. of cortisone. These findings are in keeping with the assumptions that 1) the 24-hour periodicity in the relative specific activity of phospholipid phosphorus from mouse liver cytoplasm (1, 2, 3), which is obliterated by adrenalectomy is largely dependent upon the periodic secretion of adrenal hormones, that 2) the cortex plays a more critical role in this connection than the medulla, and that 3) among the corticoids, 11-oxysteroids are more critical than 11-desoxysteroids. If theses assumptions are valid, the mechanisms of the 24-hour periodicity in the relative specific activity of these compounds may, in part, be comparable to those of the eosinophil rhythm. The latter, also, is inhibited by cortical adrenal insufficiency in mice (4, 5) and in human beings (6, 7) and also, seems largely dependent upon periodic 11-oxycorticosteroid secretion, in several species (7, 8).

An ACTH group was included in this study primarily as an additional control group. ACTH effects in adrenalectomized mice were not anticipated, unless regeneration of adrenal rests or hypertrophy of abundant ectopic cortical tissue had occurred. As indeed, no significance was associ-

ated with the ACTH treatment, it may be assumed that the possible contributions of endogenous sources of corticoid did not appreciably affect the conclusions of this study.

It also seems pertinent, that these studies were aimed at the analysis of certain mechanisms of physiologic variation (8). Other recent work on epinephrine and corticoids in relation to phospholipid metabolism has been carried out primarily from a patho-physiologic viewpoint (9, 10, 11).

SUMMARY

Groups of adrenalectomized male CBC mice, 6–8 weeks of age, kept under standardized environmental conditions were sacrificed two hours following the injection of radiophosphorus. Four hours prior to sacrifice, the mice were given certain hormone treatments or saline solution. Upon sacrifice, the livers were removed, homogenized and processed in order to determine the specific activity of phospholipid phosphorus expressed as per cent of that of acid-soluble phosphorus in a cytoplasmic fraction of the homogenate. Those groups receiving 0.9% saline, 5 μg. of epinephrine or 400 μg. of ACTH had roughly comparable values, which were below the range of physiologic variation, established earlier for intact and sham-operated mice. The relative specific activity of phospholipid phosphorus was raised to values within the physiologic range, by the administration of cortisone in 5 μg. doses. A more significant rise was brought about by 500 μg. doses of cortisone. A slight effect of desoycorticosterone, in 500 μg doses, compared unfavorably with the effect of cortisone and is not necessarily of physiologic significance. The findings support the suggestion that periodic 11-oxycorticosteroid secretion may underlie the 24-hour periodicity in cytoplasmic phospholipid metabolism of mouse liver.

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THE HYPOGLYCEMIC AND INSULINASE INHIBITORY ACTION OF L-TRYPTOPHAN[1]

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THE inactivation and degradation of insulin by the intact animal (1) and by homogenates and slices of liver (2, 3, 4) is dependent upon the activity of an enzyme system, insulinase, which is relatively specific in catalyzing the hydrolysis of insulin (5, 6). The action of insulinase can be competitively inhibited both *in vitro* (7) and *in vivo* (8) by a non-protein fraction of liver. This fraction, which appears to be a peptide, is referred to tentatively as "insulinase-inhibitor."

During the course of studies on the purification and identification of "insulinase-inhibitor," it became pertinent to determine the effect of a variety of synthetic peptides on the destruction of insulin by liver homogenates. These studies led to the observation that carbobenzoxy-l-tryptophan is a potent competitive inhibitor of insulinase *in vitro* whereas other carbobenzoxyamino acids are ineffective. L-tryptophan, however, was found to exert only a very weak inhibitory activity *in vitro* due to its rapid destruction by the liver extract. Various considerations about the mechanism whereby the carbobenzyoxy group enhanced the insulinase inhibitory activity of l-tryptophan *in vitro* led to the study of the action of this amino acid in the intact rat.

METHODS

The first series of experiments consisted of studies on the effect of the subcutaneous or oral administration of l-tryptophan and other amino acids on the blood sugar concentration of normal and alloxan diabetic rats. The amino acids were dissolved in 0.5% sodium bicarbonate, and the solution was adjusted to pH 8 and a concentration which permitted the administration of a dosage of from 1 to 4 m*M* per kilogram of body weight in 4 ml. per 100 gm. of body weight for subcutaneous injection and in 5 ml. per 100 gm. for oral administration per stomach tube.

Alloxan diabetes mellitus was produced in rats by the subcutaneous injection of 180 mg. alloxan (5% solution) per kilogram of body weight. Forty-eight hours thereafter, those animals which developed a significant glycosuria were started on a maintenance

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dose of 1 unit protamine zinc insulin[3] per day. Approximately 1 month later the insulin was discontinued for 2 days before the animals were used in this study. Only those animals which did not show an appreciable decrease in the blood sugar concentration after an overnight fast were used in this study.

After an overnight fast, groups of from 10 to 20 male rats weighing more than 120 gm. were given a solution of an amino acid or of the 0.5% sodium bicarbonate. Immediately before and at hourly intervals for five hours after the administration of the solution, blood samples were taken into 0.1 ml. pipettes from the cut tail. The glucose concentration was determined by Nelson's procedure (9).

In the second series of experiments, normal rats were given a subcutaneous injection of 4 m*M* l-tryptophan per kilogram body weight and groups of ten rats were sacrificed immediately and at hourly intervals for four hours after the injection. Each rat's liver was removed, homogenized with four volumes of 0.02 *M* sodium bicarbonate, centrifuged for 30 minutes at 18,000×g. and 4° C, the supernatant removed, adjusted with *M*/15 phosphate buffer to pH 7.8 and the insulinase activity determined as described previously (5). The activity of the supernatant was expressed in terms of the protein concentration which was determined by the procedure of Lowry, Rosebrough, Farr and Randall (10).

In the third series of experiments, the effect of the subcutaneous injection of l-tryptophan on the destruction of insulin by intact mice was determined. Groups of fed male mice were given a subcutaneous injection of 0.004 m*M* l-tryptophan in a volume of 0.04 ml. per gram of body weight. One hour later, one group of 10 mice was given an intraperitoneal injection of 0.4 units insulin in 0.02 ml. acidified water per gram of body weight and a subcutaneous injection of 1 ml. of a 5.5% glucose solution. The insulin consisted of a mixture of crystalline zinc insulin and tracer quantities of I^{131} labeled insulin.[4] A second group of mice was treated similarly but was given the injection of insulin two hours after the administration of the l-tryptophan. The third group of mice was given the insulin in three hours after the l-tryptophan. All mice were sacrificed one hour after the injection of the insulin. A fourth group of mice which was given sodium bicarbonate instead of l-tryptophan served as controls. The preparation of the animals and the determination of the quantity of insulin destroyed per hour per 100 gm. of mouse was the same as that described in a previous report (1).

In addition to the above, 3 groups of mice were given a subcutaneous injection of the same quantity of l-tryptophan or of the 0.5% sodium bicarbonate and one hour later were injected intraperitoneally with 0.02, 0.04 and 0.4 units of insulin per gram of body weight, and subcutaneously with 1 ml. of 5.5% glucose. Each dose of insulin contained the same quantity of the I^{131} labeled insulin. One hour after the injection of the insulin, the mice were sacrificed and the quantity of insulin destroyed by each animal in the interval was determined.

RESULTS

Hypoglycemic Response

The data on the effect of the subcutaneous injection of various concentrations of l-tryptophan on the blood sugar of fasted rats are summarized in Table 1. In order to permit comparison between the various groups, the

[3] We are indebted to Dr. W. R. Kirtley of the Eli Lilly Company for generous supplies of insulin.

[4] We are indebted to Dr. F. J. Dixon of the Department of Pathology, University of Pittsburgh, School of Medicine, for the preparation of the I^{131} labeled insulin

TABLE 1. EFFECT OF THE SUBCUTANEOUS INJECTION OF L-TRYPTOPHAN ON BLOOD SUGAR. L-tryptophan or sodium bicarbonate injected in volume of 4 ml. per kilogram of body weight immediately after the initial blood sample. All values expressed as mean ±S.E.

Dose per kilo	Number of rats	Weight, grams	Blood sugar—mgm.%					
			0	1 hr.	2 hrs.	3 hrs.	4 hrs.	5 hrs.
Sodium Bicarbonate—0.5% solution								
40 ml.	10	178.6±3.3	77.4±3.6	74.2±3.4	72.2±4.0	78.4±3.4	81.6±4.5	80.4±4.0
L-Tryptophan								
1 mM.	10	252.1±7.5	76.8±5.1	74.0±4.9	62.5±4.0	63.1±5.3	73.4±5.2	76.8±4.1
4 mM.	10	256.2±8.6	73.6±1.9	75.2±1.2	70.0±2.3	60.4±2.4	62.3±2.8	60.6±5.4

blood sugar concentration for each interval after the administration of l-tryptophan was computed as the per cent of the initial blood sugar concentration and the mean for each group plotted in Figure 1. It is apparent that in contrast to the effect of sodium bicarbonate, the subcutaneous injection of even 1 m*M* of l-tryptophan per kilogram of body weight produced a decrease in the concentration of the blood sugar which was statistically significant by the third hour ($P < 0.02$). The injection of 4 m*M* l-tryptophan produced a more marked ($P < 0.001$) and more prolonged decrease in the blood sugar. The statistically highly significant difference ($P < 0.001$)

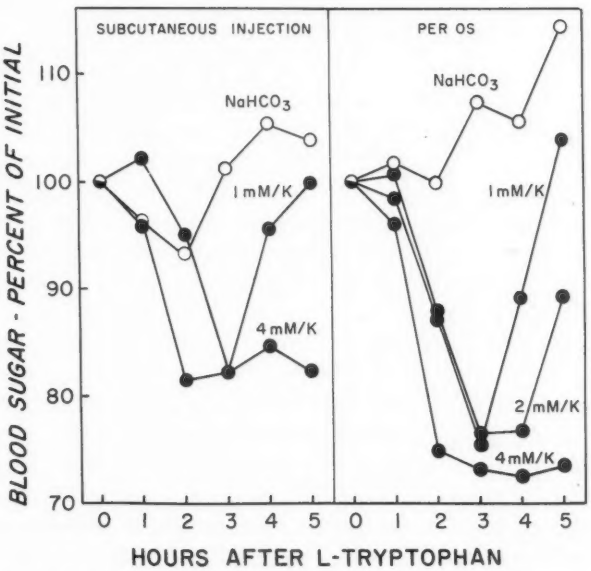

FIG. 1. Effect of the subcutaneous or oral administration of l-tryptophan on the blood sugar. The blood sugar concentration at each interval is expressed as the per cent of that immediately before the administration of the amino acid.

between the effect of 1 m*M* and 4 m*M* of l-tryptophan per kilogram body weight was revealed also by a repeated measures analysis of variance of the two curves (11).

The administration of l-tryptophan per stomach tube resulted in a highly significant decrease in the blood sugar concentration in from 2 to 3 hours ($P < 0.001$) at all dosage levels used (Table 2, Fig. 1). The larger doses, however, induced a greater prolongation of the hypoglycemia. Moreover, the response to the oral administration was greater than that to similar quantities of l-tryptophan administered subcutaneously ($P < 0.001$). Thus, whereas the subcutaneous injection of 4 m*M* per kilogram resulted in a maximum depression of 17.9% from the initial blood sugar concentration, the same quantity by stomach tube resulted in a drop of 26.8% from the initial concentration. The difference in the response to the two routes of administration of l-tryptophan is revealed also in the fact that mean decrease of the blood sugar over the five hour period was 10.7 and 21.9% for the subcutaneous and oral routes respectively. The statistically highly significant difference ($P < 0.001$) between the blood sugar response to l-tryptophan by the two routes was revealed also by a repeated measures analysis of variance of the two responses.

TABLE 2. EFFECT OF L-TRYPTOPHAN PER OS ON BLOOD SUGAR.
L-tryptophan or sodium bicarbonate was administered by stomach tube in a volume of 5 ml. per 100 grams of body weight immediately after the initial blood sample.
All values expressed as mean ±S.E.

Dose	Number of rats	Weight, gms.	Blood sugar—mgm.%					
			0	1 hr.	2 hrs.	3 hrs.	4 hrs.	5 hrs.
Sodium Bicarbonate								
50 ml./K.	20	174.1±3.9	75.6±2.7	76.9±2.6	75.5±2.3	81.2±1.8	79.8±2.4	86.5±3.6
L-Tryptophan								
1 mM./K.	24	196.9±7.9	79.5±2.2	78.2±2.2	69.3±1.7	60.1±2.7	71.0±3.5	82.7±3.4
2 mM./K.	19	181.2±7.0	74.1±2.5	74.6±1.7	65.2±2.4	56.8±2.7	57.0±3.7	66.2±3.9
4 mM./K.	22	164.0±5.8	85.7±3.8	82.3±3.9	64.3±2.5	62.7±3.6	62.2±4.5	63.0±5.3

In order to determine whether the hypoglycemic action of l-tryptophan is specific to this amino acid, seventeen other amino acids, including d-tryptophan, were administered by stomach tube to groups of 10 rats. Each amino acid was given in a dosage of 4 m*M* per kilogram of body weight. Whereas the administration of l-tryptophan resulted in a statistically highly significant decrease in the blood sugar concentration ($P < 0.001$), none of the other amino acids produced a significant decrease while some of them produced a significant hyperglycemia (Table 3). Of particular interest is the fact that d-tryptophan produced no significant change in the blood sugar from that of the sodium bicarbonate treated animals.

During the course of these studies it became apparent that very young rats showed a greater hypoglycemic response to the administration of 4

TABLE 3. EFFECT OF VARIOUS AMINO ACIDS PER OS ON BLOOD SUGAR.
The amino acids were administered per stomach tube in a dosage of 4mM per kilogram body weight immediately after the initial blood sample. All values expressed as mean ±SE

Amino acid	Weight, gm.	Blood sugar—mgm.%						Mean change, %
		0	1 hr.	2 hrs.	3 hrs.	4 hrs.	5 hrs.	
L-Tryptophan	164.0± 5.8	85.7±3.8	82.3± 3.9	64.3±2.5	62.7±3.6	62.2±4.5	63.0±5.3	−21.9
L-Lysine HCL	200.0± 4.6	75.3±2.2	78.1± 3.6	71.8±3.8	79.4±3.0	71.5±3.0	72.5±3.1	−0.9
L-Cystine	211.8± 5.7	75.1±2.5	76.8± 2.2	70.5±2.2	71.9±3.3	80.1±2.2	82.0±4.0	+1.6
L-Isoleucine	181.1± 2.8	63.5±2.3	57.0± 1.6	58.3±2.4	63.6±3.1	72.1±2.6	72.0±2.3	+2.4
L-Threonine	175.7± 4.0	70.4±3.2	75.0± 3.5	67.0±4.0	75.4±4.3	73.3±4.9	79.7±4.2	+5.2
D-Tryptophan	270.1± 8.1	83.4±4.7	84.7± 3.6	83.6±5.9	87.3±3.6	91.2±5.2	100.4±3.0	+7.2
L-Aspartic Acid	222.0± 6.2	75.9±3.0	87.7± 4.5	80.5±4.0	82.8±5.5	77.7±4.9	78.9±4.7	+7.4
L-Leucine	168.3± 4.7	70.0±5.6	65.6± 4.5	75.0±8.0	79.8±5.5	74.0±2.3	81.8±5.4	+7.5
L-Arginine HCL	177.5± 1.9	67.7±2.5	73.7± 2.5	67.5±1.3	74.2±4.4	73.0±4.8	76.7±3.9	+7.9
Glycine	235.0±10.8	59.6±5.4	63.2± 2.0	62.2±5.9	67.4±6.8	60.0±2.1	69.2±2.7	+8.1
L-Histidine HCL	171.3± 3.0	64.5±3.7	65.1± 2.7	65.8±3.5	71.2±3.9	74.4±3.5	77.8±4.1	+11.0
L-Sarine	174.5± 2.3	68.4±2.2	78.3± 1.5	76.4±0.7	78.3±1.9	77.7±2.3	76.5±2.3	+11.2
L-Methionine	175.4± 6.7	66.1±2.0	74.8± 2.5	76.4±4.2	72.9±4.2	71.6±5.5	73.8±4.1	+11.2
L-Tyrosine	171.7± 3.5	65.6±1.7	73.9± 3.1	75.5±3.5	74.5±2.8	76.5±2.5	75.5±2.2	+11.5
L-Alanine	164.5± 4.4	73.0±3.6	88.9± 3.2	80.5±2.8	81.7±3.8	77.3±3.0	79.2±2.3	+11.7
L-(+)-Glutamic Acid	218.3±13.8	72.2±3.7	93.2± 3.4	83.2±4.0	74.6±3.4	81.1±1.9	82.2±1.8	+14.8
L-Valine	168.5± 6.0	61.0±1.5	70.4±10.9	75.0±7.0	74.4±6.1	77.6±7.6	77.4±3.0	+22.9
L-Phenylalanine	162.0± 4.4	75.0±2.0	89.5± 3.0	87.5±3.3	93.0±3.3	100.0±3.7	101.1±2.5	+25.6

m*M* l-tryptophan per kilogram body weight. Accordingly, three groups of 10 rats of a mean ±SE weight of 76.4±1.7 gm., 145.5±2.0 gm. and 329.0 ±3.3 gm. were given 4 m*M* l-tryptophan per kilogram of body weight and the blood sugar response studied as described. Figure 2 illustrates the mean response of the three groups of rats and reveals that whereas there was no difference in the hypoglycemic response of the two groups consisting of animals weighing more than 120 gm., there was statistically highly significantly greater prolongation of the depression in the blood sugar of the very young rats weighing less than 120 gm. ($P < 0.001$).

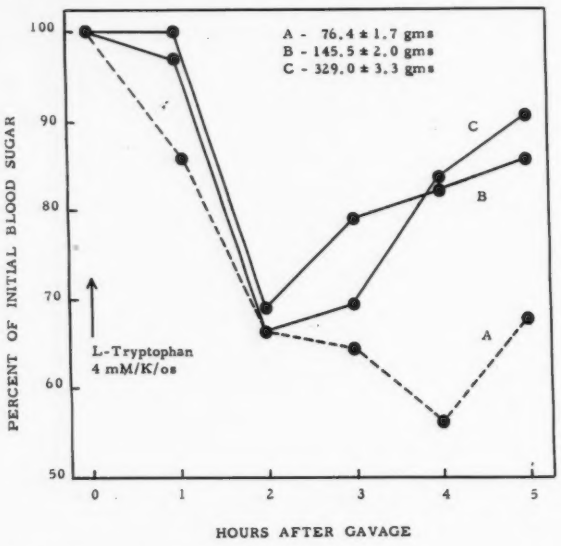

FIG. 2. Effect of l-tryptophan by mouth on blood sugar response of young and adult rats. The blood sugar concentration at each interval is expressed as the per cent of that immediately before the administration of the l-tryptophan per stomach tube.

In order to determine whether the hypoglycemic response to l-tryptophan is dependent upon the availability of endogenous insulin, the amino acid was administered to a group of 15 severely diabetic alloxanized rats after an overnight fast. The mean ± SE initial blood sugar concentration of the group was 309±26 mg. %. It is apparent from Figure 3 that the administration of l-tryptophan by mouth did not decrease the blood sugar concentration of the diabetic rats. Similar results were obtained when the l-tryptophan was injected subcutaneously.

Insulinase Activity

The effect of the oral administration of 4 m*M* l-tryptophan per kilogram body weight on the insulinase activity of the liver is illustrated in Figure 4 where the mean ± SE of the insulinase activity of the liver extract is de-

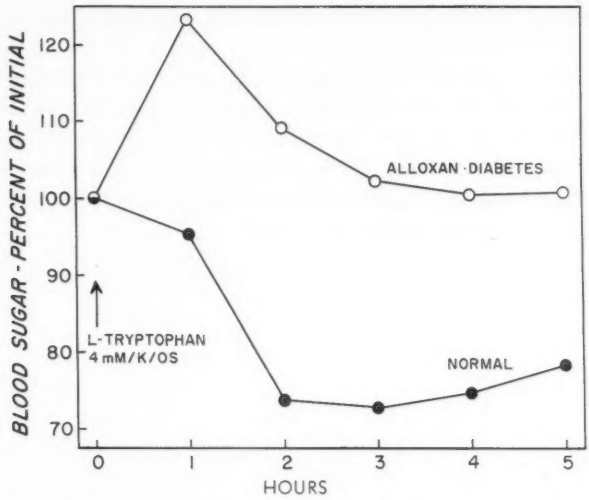

FIG. 3. Effect of l-tryptophan by mouth on the blood sugar response of alloxan diabetic rats. The blood sugar concentration at each interval is expressed as the per cent of that immediately prior to the administration of the l-tryptophan per stomach tube. The mean ±SE initial blood sugar concentration of the diabetic group was 309±26 mgm. per cent.

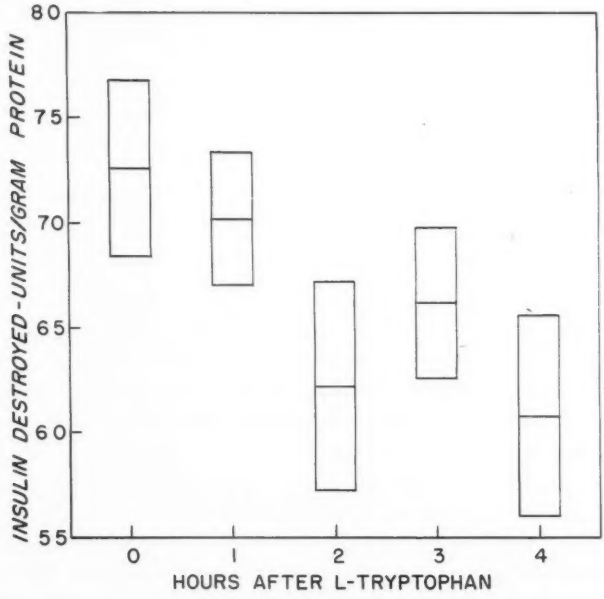

FIG. 4. Effect of l-tryptophan by mouth on the insulinase activity of rat livers. The insulinase activity is expressed as the mean ±SE units of insulin destroyed per gram of protein in the liver extract (see text).

picted in terms of the quantity of insulin destroyed *in vitro* in 30 minutes per gram of protein. A statistically highly significant decrease in the insulinase activity occurred after the first hour ($P < 0.001$).

That the reduction in the insulinase activity of the liver is not due to an artifact related to the *in vitro* assay is indicated by the observations depicted in Figure 5. Like with the homogenates, the insulinase activity of intact mice was markedly decreased within 2 hours after the subcutaneous administration of l-tryptophan ($P < 0.001$).

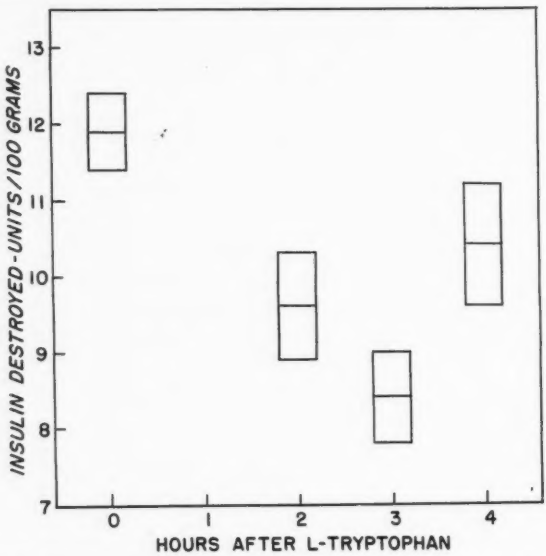

FIG. 5. Effect of l-tryptophan on the destruction of exogenous insulin by intact mice. The insulinase activity is expressed as the mean ±SE units insulin destroyed per 100 grams of mouse in one hour (see text).

To determine whether or not l-tryptophan behaves like a competitive inhibitor in the intact animal, the effect of the amino acid on the subsequent destruction of different quantities of exogenous insulin was determined. A Lineweaver-Burk (12) plot of the quantity of insulin destroyed by each group of 10 mice (Figure 6) suggests that l-tryptophan behaved as a competitive inhibitor of insulinase.

DISCUSSION

The data reported herein reveal that l-tryptophan is relatively specific in the production of a statistically significant decrease in the blood sugar concentration of normal rats. In accord with these observations are those of Gullino *et al.* (13) who found that rats injected intraperitoneally with a LD 99.99 dose of l-tryptophan (12 m*M*/kg.) died with marked hypogly-

cemia. This hypoglycemic response to a toxic dose of l-tryptophan was preceded by a marked hyperglycemia. In the present studies, however, the hypoglycemic response to the subcutaneous or oral administration of nontoxic doses of l-tryptophan was not preceded by a rise in the blood sugar concentration.

A small but significant hypoglycemic response to the ingestion of dl-tryptophan was reported by Turner and Crowell (14) but refuted by How-

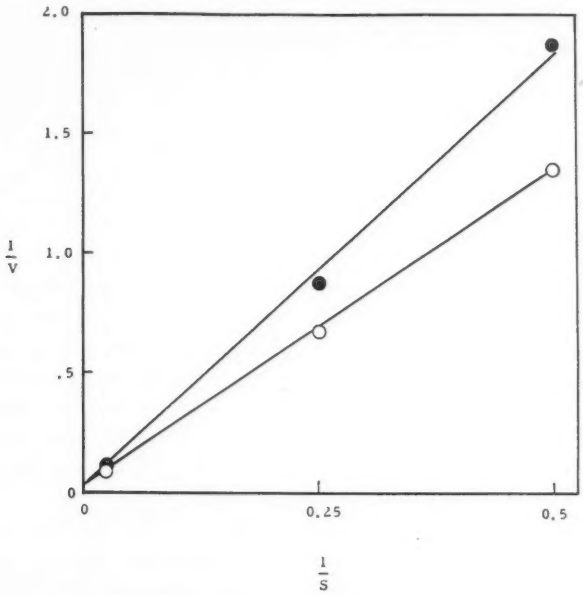

FIG. 6. Effect of l-tryptophan on destruction of various quantities of exogenous insulin by mice. All animals received a subcutaneous injection of sodium bicarbonate (○) or 0.4 m*M* l-tryptophan per 100 grams body weight (●) one hour before injection of I^{131} labeled insulin. Each point represents mean of 10 mice. V = units I^{131} labeled insulin destroyed per 100 grams of mouse in 60 min. S = units I^{131} insulin injected intraperitoneally per 100 grams of mouse.

ard and Modlinger (15) and by Mann *et al.* (16). More recently, Mellinkoff *et al.* (17) reported a significant hypoglycemic response in man to the slow infusion or ingestion of an enzymatic digest of casein supplemented with l-tryptophan. It is quite possible that this hypoglycemic response was due to the action of the l-tryptophan. However, the marked hypoglycemic response of some patients with idiopathic familial hypoglycemia to the ingestion of protein is attributed by Cochrane *et al.* (18) to leucine and isovaleric acid. Since Gullino *et al.* (13) observed that rats given LD 99.99 doses of isoleucine, alloisoleucine, valine, leucine and phenylalanine also died with hypoglycemia, it is quite possible that a combination of the various amino

acids in non-toxic doses may act as l-tryptophan in reducing the blood sugar concentration.

The administration of l-tryptophan to the fasted severely diabetic alloxanized rats did not result in a decrease in the blood sugar concentration. Since the alpha cells of the Islets of Langerhans of alloxanized rats are relatively intact, it may be concluded that the hypoglycemic response of normal rats to l-tryptophan is not due to a decrease in the production or the activity of glucagon. Likewise, the hypoglycemic response of the normal rat cannot be due to some acute non-specific hepatotoxic action of l-tryptophan since such action would occur in the alloxanized as well as the normal rat. Consequently, it may be concluded that the hypoglycemic response of the normal animal is due to an increase in the availability of insulin.

An increase in the availability of insulin may result from an increased secretion of insulin consequent to stimulation of the beta cells of the Islets of Langerhans or to a decrease in the destruction of endogenous insulin consequent to an inhibition of the enzyme which catalyzes the hydrolysis of insulin (insulinase), or to a combination of both mechanisms. The long interval between the injection or ingestion of the amino acid and the maximum hypoglycemic response does not favor the possibility that the response is due to an increase in the secretion of insulin. On the other hand, the marked decrease in the insulinase activity of livers taken from l-tryptophan treated rats and the marked decrease in the rate of destruction of insulin by similarly treated mice favor the hypothesis that the hypoglycemic response to the amino acid is due, in part at least, to a decrease in the rate of destruction of endogenous insulin.

The greater hypoglycemic response of very young rats (below 120grams body weight) to the administration of l-tryptophan may be related to a relatively greater rate of insulin secretion by such animals. Direct observations on the quantity of insulin secreted at various age periods are not available. The fact that insulin is essential for growth, and the observation that young animals do not succumb to the diabetogenic action of anterior pituitary extracts (19) suggest that such animals may be capable of producing much larger quantities of insulin than do adult animals. If the above be true, then an inhibition of the rate of endogenous insulin destruction will result in a greater hypoglycemia in the young than in the adult rat as observed herein after the administration of l-tryptophan.

The demonstration that the injection of increasing quantities of insulin overcomes the inhibition of insulinase induced by a constant quantity of l-tryptophan (Fig. 6) suggests that the amino acid behaves *in vivo* like a competitive inhibitor of insulinase. In this regard, the response to l-tryptophan is similar to the competitive inhibition of insulinase which is exerted *in vitro* and *in vivo* by a peptide-like hepatic factor which has been tentatively designated as "insulinase inhibitor" (8). The fact that l-tryptophan exerted only a weak inhibitory action on insulinase *in vitro* suggests

that some metabolic product of the amino acid may be responsible for the effect noted *in vivo*. In accord with this hypothesis is the observation that indole-3-acetic acid and some other derivatives of l-tryptophan are effective in producing hypoglycemia and insulinase inhibition in normal rats (20).

SUMMARY AND CONCLUSION

The administration of from 1 to 4 m*M* of l-tryptophan per kilogram of body weight resulted in a marked reduction in the blood sugar concentration of normal but not of severely diabetic alloxanized rats. Concomitantly, there was a marked reduction in the insulinase activity of extracts of livers removed from normal rats at intervals after the ingestion of l-tryptophan. Likewise, the administration of l-tryptophan inhibited the destruction of exogenous insulin by intact mice. Accordingly, the hypoglycemic response to l-tryptophan is attributed to an increase in the availability of insulin consequent to a decrease in the destruction of endogenous insulin.

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NOTES AND COMMENTS

PROTECTION BY ALDOSTERONE AND 11,17-OXYCORTICOIDS AGAINST EFFECTS OF BRUCELLA SOMATIC ANTIGEN IN ADRENALECTOMIZED MICE[1,2]

Cortisone and 9α-fluorohydrocortisone protect against the hypothermia induced by Brucella somatic antigen (endotoxin) in adrenalectomized mice (1). The possibility that aldosterone may act similarly was explored herein. Moreover, in this study, the prolongation by aldosterone of the survival time of adrenalectomized mice given endotoxin also was examined.

Groups of CBC and D_8BC mice, males, 6–8 weeks of age were studied, under the experimental circumstances described by Halberg and Spink (1). At variance with the earlier procedure, however, the temperatures of the mice were measured prior to injection as well as at different time points after injection. The materials examined were aldosterone in purified sesame oil,[3] purified sesame oil,[3] crystalline aldosterone, hydrocortisone (free alcohol), cortisone (free alcohol), 9α-fluorohydrocortisone in alcohol and saline suspensions of 9α-fluorohydrocortisone acetate and of cortisone acetate (Cortone-Merck). Unless otherwise indicated, the corticoids were given intraperitoneally, in the same injection with 0.2 mg. of endotoxin, and in 0.2 cc. of total volume. The doses were adjusted for the body weight of each mouse; they are listed as per 20 gm. of body weight.

RESULTS

Figure 1 shows the results of the first experiment with aldosterone. The compound was given in purified sesame oil in $2\frac{1}{2}$ μg. doses, by the intraperitoneal route to one group of mice and by the intramuscular route to another group of mice. The measurements of temperature were extended over a 500 minute post-injection period because a slow absorption of the compound in oil may be associated with delayed effects. It is seen from Figure 1, however, that in doses of $2\frac{1}{2}$ μg., aldosterone did not protect against the hypothermia induced by the endotoxin. The temperature curves of the groups receiving aldosterone intramuscularly or intravenously are roughly comparable to those of the corresponding two control groups receiving endotoxin and purified sesame oil (without aldosterone) as well as to the temperature curve of the group receiving only endotoxin. It also may be seen from Figure 1 that at the $2\frac{1}{2}$ μg. dose level, both cortisone acetate and 9α-fluorohydrocortisone acetate protect against hypothermia, the latter compound being again decidedly more active than the former.

Figure 2 shows the activity of aldosterone in 1.3% alcohol at dose levels of 5 and 15 μg., and further reveals that in 5 μg. doses all of the 11,17-oxycorticoids studied, i.e. cortisone, hydrocortisone and 9α-fluorohydrocortisone compare favorably with aldo-

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[2] The authors acknowledge gratefully the technical assistance of Paul Albrecht.

[3] Kindly supplied by Dr. Robert Gaunt of Ciba Pharmaceutical Products, Inc., Summit, New Jersey.

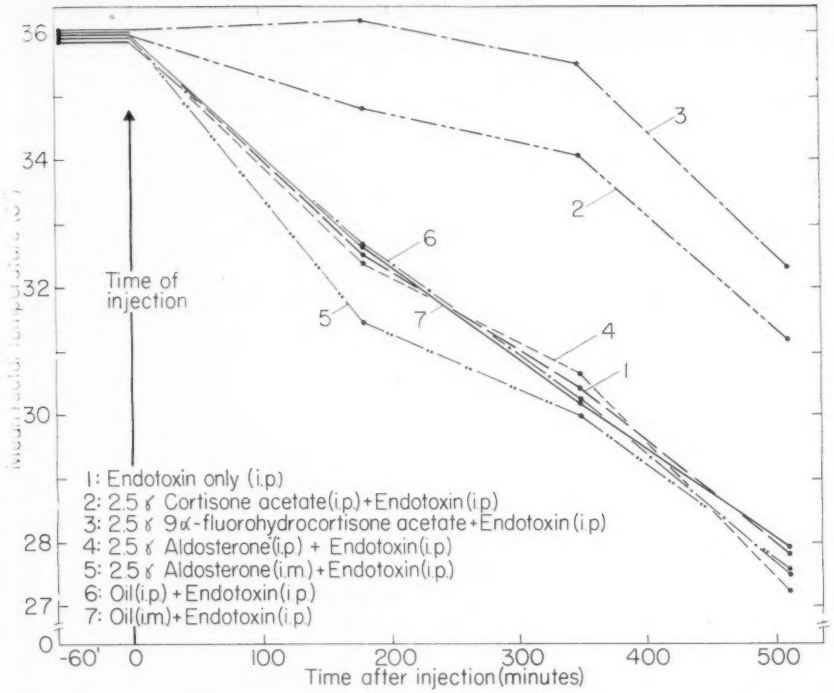

FIG. 1. Anti-hypothermic activity of $2\frac{1}{2}$ μg. doses of 11,17-oxycorticoids and lack of effects of $2\frac{1}{2}$ μg. of aldosterone in purified sesame oil, 9 mice per group.

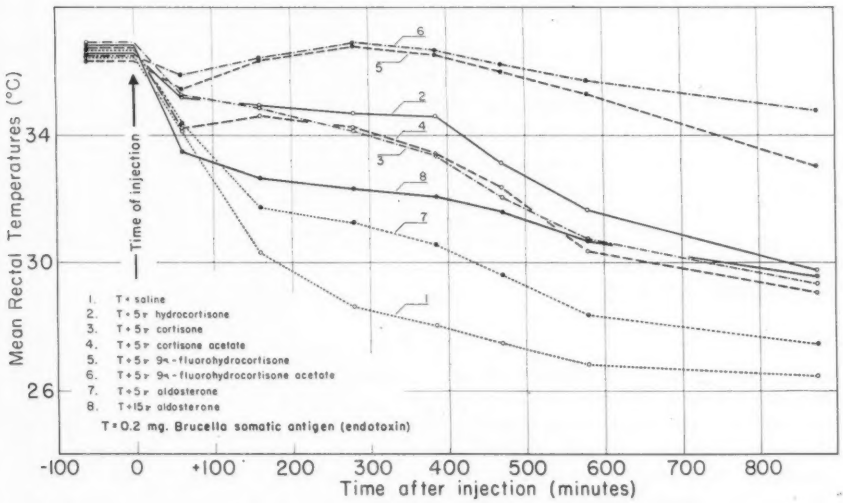
Mean Rectal Temperatures (°C)
34
30
26
Time of injection
1. T + saline
2. T + 5γ hydrocortisone
3. T + 5γ cortisone
4. T + 5γ cortisone acetate
5. T + 5γ 9α-fluorohydrocortisone
6. T + 5γ 9α-fluorohydrocortisone acetate
7. T + 5γ aldosterone
8. T + 15γ aldosterone
T = 0.2 mg. Brucella somatic antigen (endotoxin)
-100 0 +100 200 300 400 500 600 700 800
Time after injection (minutes)

FIG. 2. Comparison of anti-hypothermic effects of various oxycorticoids, 10 mice per group.

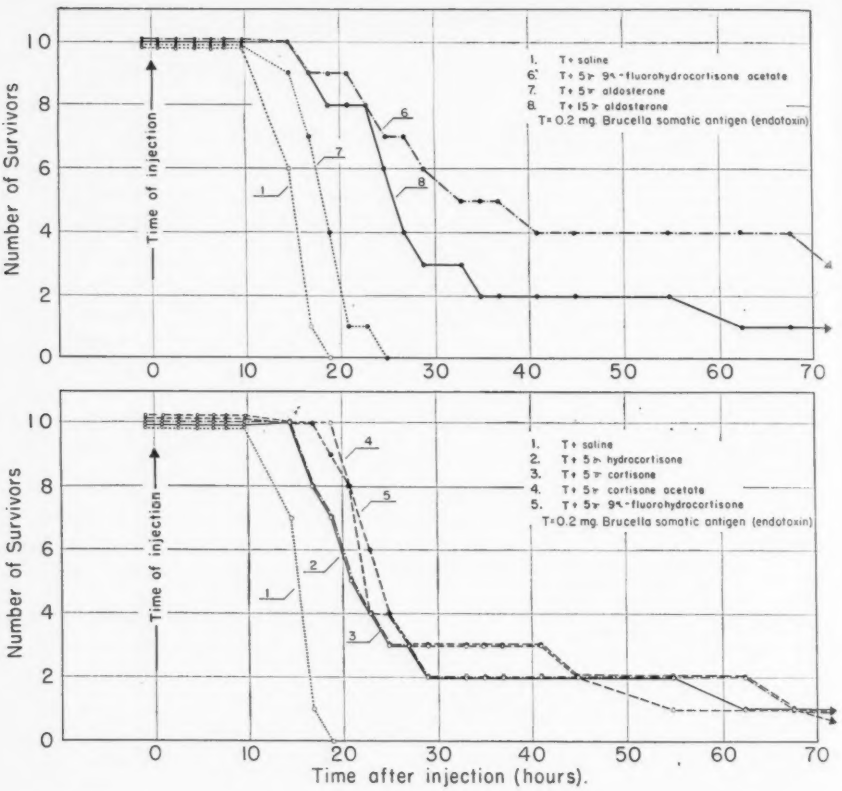
Number of Survivors
Time of injection
1. T+ saline
6. T+ 5γ 9α-fluorohydrocortisone acetate
7. T+ 5γ aldosterone
8. T+ 15γ aldosterone
T=0.2 mg. Brucella somatic antigen (endotoxin)
1. T+ saline
2. T+ 5γ hydrocortisone
3. T+ 5γ cortisone
4. T+ 5γ cortisone acetate
5. T+ 5γ 9α-fluorohydrocortisone
T=0.2 mg. Brucella somatic antigen (endotoxin)
Time after injection (hours).

FIG. 3. Prolongation by corticoids of survival time of adrenalectomized male D_8BC mice given Brucella somatic antigen: not more than one animal per group survived at 90 hours after injection. (10 mice per group; same mice as in Fig. 2).

sterone. The activity of these 11,17-oxycorticoids seems to increase in the order of listing. The survival times of the same treatment-groups of mice are exhibited in Figure 3. An effect of 5μg. of aldosterone in prolonging survival is barely detectable. For aldosterone in 15 μg. doses, this effect is more pronounced. The several 11,17-oxycorticoids again compare favorably with aldosterone, with respect to life-maintenance in the animal-preparation studied.

DISCUSSION

A variety of effects of aldosterone have recently been reviewed by Gaunt *et al.* (2). To these effects may be added the abilities of aldosterone (in alcohol) 1) to protect against the hypothermia of adrenalectomized mice given endotoxin and 2) to prolong the survival time of the same animal-preparation. These effects were seen at the 5μg. /20 gm. of body weight dose-level and they may have physiologic significance. Aldosterone in oil was found inactive, but it was tested at a lower dose level. Thus, possible differences between the effects of the compound in oil and in alcohol can not be assessed in this study. Furthermore, aldosterone is merely one of several steroidal compounds

which all exhibit the biologic effects studied to a degree which seems to be related to chemical structure. Other things being constant, it appears that 11,17-oxycorticoids are more potent than is the 11, 18-oxycorticoid aldosterone.

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THE ENDOCRINE SOCIETY

1957 Annual Meeting

The Thirty-ninth Annual Meeting of The Endocrine Society will be held in the Hotel New Yorker, New York, New York, Thursday, Friday, and Saturday, May 30, 31, June 1, 1957.

The Committee on Local Arrangements is Dr. Rulon W. Rawson as Chairman with Drs. Earl T. Engle, Joseph W. Jailer, Warren O. Nelson, and Martin Sonenberg as members of the Committee.

All Scientific Sessions will be held in the Hotel New Yorker. The rooms in which each session will be held will be announced in the program and on the hotel bulletin board. The Annual Dinner is scheduled for Friday, May 31, at 7:30 p.m., preceded by cocktails at 6:30 p.m.

All members are urged to make hotel reservations immediately inasmuch as the hotels expect to be filled to capacity. Make reservations directly with the New Yorker, advising time of arrival and departure date. Make your reservations now and avoid disappointment.

Those wishing to present papers, which will be strictly limited to ten minutes, should send four copies of the title and abstract to the Vice-President, Dr. Eleanor Venning, Royal Victoria Hospital, Montreal, Quebec, Canada, not later than February 1, 1957. *It is imperative that the abstracts be informative and complete with results and conclusions—not a statement that these will be presented at the meeting*—in order that they may be of reference value and suitable for printing in the program and Journals of the Society. The following regulations for the preparation of abstracts and titles must be carefully followed to insure consideration of the paper for the program:

1. Abstracts may not exceed two hundred words, or equivalent space, exclusive of title. No footnotes or acknowledgments to sponsors can be published. Reference, if used, must be placed in the body of the text. The abstract should consist of a single paragraph, if possible. Structural chemical formulas cannot be used.
2. The title heading must be arranged as follows:
   - Line 1. Title, not to exceed fifteen words.
   - Line 2. Author/s. The name of each non-member author collaborating with member-authors is to be followed by the phrase "(by invitation)." Names of non-members who are introduced, i.e., who are not collaborators with member-authors, are to be followed by the phrase "(introduced by . . .)." The principal degree, e.g., M.D., of each author should be given after his name.
   - Line 3. Institution of origin and city in which institution is located.

3. The body of the abstract, typed double-space, should follow the heading. The original copy should be on bond paper. There should be three copies.
4. Abstracts should be letter perfect, since there will be no opportunity for proof reading by authors.

1957 AWARDS

The selection of the recipients of the awards of The Endocrine Society is made by a Committee appointed by the Council of the Society. These awards and fellowships carry no obligation by the recipient to the Society or to the donors.

Medal of The Endocrine Society

In 1954 the Council of the Society voted to establish a medal and an honorarium of $1,000 to be given to an individual for work of special distinction in endocrinology. The recipient shall be chosen from nominations presented by members of the Society and is limited to citizens of the United States and Canada. Dr. Carl R. Moore was the recipient in 1955 and Dr. Frederick L. Hisaw in 1956.

The Ciba Award

The Ciba Award, to recognize the meritorious accomplishments of an investigator not more than 35 years of age in the field of clinical or preclinical endocrinology, was established in 1942, but no recipient was selected in 1942 or 1943. In 1944 the Award was presented to Dr. E. B. Astwood; 1945—Dr. Jane A. Russell; 1946—Dr. Martin M. Hoffman; 1947—Dr. Choh Hao Li; 1948—Dr. Carl Heller; 1949—Dr. George Sayers; 1950—Dr. Oscar M. Hechter; 1951—Dr. Albert Segaloff; 1952—Dr. Seymour Lieberman; 1953—Dr. Sidney Roberts and Dr. Clara M. Szego (Mrs. Roberts); 1954—Dr. I. N. Rosenberg; 1955—Dr. Jack Gross; 1956—Dr. Alfred M. Bongiovanni. Prior to 1952 the Award was $1,200. It is now $1,800. If within twenty-four months of the date of the award, the recipient should choose to use it toward further study in a laboratory other than that in which he is at present working, it will be increased to $2,500.

The Ayerst and the Squibb Fellowships

The Ayerst Fellowship was established in 1947 and the Squibb Fellowship in 1956. They are designed to assist men or women of exceptional promise in furthering their advancement towards a career in endocrinology. Each Fellowship is awarded on alternate years and the stipend, which will not exceed $5,000, may be divided into two Fellowships in varying amounts

in accordance with the qualifications of the appointees. Individuals possessing the M.D. or Ph.D. degree, or candidates for either of these degrees are eligible for appointment.

Applicants must submit the following information:

1. Evidence of scientific ability as attested by studies completed or in progress.
2. Recommendations from individuals familiar with the candidate and his work.
3. A proposed program of study.
4. Acceptance of the individual by the head of the department in which the Fellowship will be held.
5. A statement that he or she will serve full time if awarded a Fellowship. A small amount of time (10 to 15 per cent) may be spent in course work or participation in teaching, the latter purely on a voluntary basis.

The Schering and The Upjohn Scholars of The Endocrine Society

The Council of The Endocrine Society has established a category of Scholars. These grants have been made available through the generosity of the Schering Corporation and the Upjohn Company, and will be awarded to established investigators and teachers in the field of endocrinology who wish to extend their opportunities for work either in this country or abroad.

The award will not exceed $2,500 annually for each individual and shall be granted on the basis of proposals submitted by the applicant. Such applications should include the estimated financial needs. The funds may be used for travel, maintenance and other expenses.

Nominations

Nominations for the Medal of the Endocrine Society; the Ciba Award; the Ayerst and the Squibb Fellowships may be made by any member of The Endocrine Society. They should be submitted on forms which may be obtained from the Office of the Secretary, 1200 North Walker Street, Oklahoma City 3, Oklahoma. Completed nominations should be returned to the Secretary not later than *November 1* each year.

Proposals for appointment as Scholars of The Endocrine Society should be made in writing by the individual, and addressed to the Secretary of the Society. They should be submitted by *November 1, 1956.*

The Awards Committee will meet in November and notice of awards to successful nominees and applicants will be made not later than December 1, 1956.

TRAINING PROGRAM FOR PERSONS INTERESTED IN STEROID INVESTIGATIONS

Recognizing the increasing importance of steroids in many fields of medical research, the United States Public Health Service has awarded grants making possible a program to provide specialized training for individuals interested in steroid investigation. The National Cancer Institute of the National Institutes of Health has established grants for a joint training program in steroid biochemistry to be conducted at Worcester, Massachusetts and at Salt Lake City, Utah through the cooperative effort of the Worcester Foundation for Experimental Biology with the Department of Chemistry, Clark University and the Department of Biochemistry, College of Medicine, University of Utah. Two types of fellowships are planned: a one-year program carrying a stipend of $5000, for individuals having a B.S. or M.S. degree in chemistry or in medical technology, or an equivalent, which would carry a stipend of $1500 for the six-month period. The post-doctoral fellowship is intended for scientists who contemplate research involving steroid techniques and who wish to obtain a broad experience. During a portion of the year such fellows may initiate work on their particular problems under the guidance of established investigators. The pre-doctoral program is primarily intended to train individuals in the analytic determination of various steroids. Trainees completing the six-month program will be able to carry out such determinations competently, either in connection with a research program or for diagnostic purposes. They should also be in a position to master new techniques as they appear. Both training programs will include theoretical and practical considerations as well as experience in specialized techniques. The first groups will begin January 1, 1957, at Salt Lake City, closing date for application November 1; and February 1, 1957 at Worcester, Mass., closing date for application November 1, 1956. Individuals or institutions wishing to obtain more information regarding this program should communicate directly with Dr. Kris Eik-Nes, Department of Biochemistry, College of Medicine, University of Utah, Salt Lake City or Dr. Frank Ungar, Worcester Foundation for Experimental Biology, Shrewsbury, Mass.

MEDICAL RESEARCH FELLOWSHIPS

The Division of Medical Sciences of the National Academy of Sciences-National Research Council will accept applications for postdoctoral research fellowships for 1957–1958 until December 1, 1956. The Fellowships are awarded in the early Spring. Complete details and application blanks

may be obtained from the *Division of Medical Sciences, Room 310, National Academy of Sciences-National Research Council, 2101 Constitution Avenue, N. W., Washington 25, D. C.*

A. The following groups of fellowships are awarded and administered by the Division's Medical Fellowship Board:

(1) *National Research Fellowships in the Medical Sciences,* supported since 1922 by The Rockefeller Foundation.
(2) *Donner Fellowships for Medical Research,* made possible by a new grant from the Donner Foundation.
(3) *Markle Fellowships in the Medical Sciences,* provided through a new appropriation of the John and Mary R. Markle Foundation.

The purposes and conditions governing these three programs are identical. They are designed to offer research experience in the basic medical sciences for persons who look forward to careers in academic medicine and investigation. Fellows are therefore expected to devote essentially their entire time to research at the fundamental level. Funds are not available for support of those who are seeking practical experience in the clinical field.

Awards are open to citizens of the United States and Canada who hold the M.D., Ph.D. degree, or the equivalent. Ordinarily Fellowships are not granted to persons over thirty-five years of age.

B. *Fellowships in Radiological Research* are administered for the James Picker Foundation by the Division's Committee on Radiology. Applications will be entertained from candidates seeking to gain research skills leading to investigative careers in the field of radiology. While persons from closely related disciplines are eligible to apply, candidates whose training has been directly in the field of radiology will receive preference under this program. Candidates must hold the M.D., Ph.D. or Sc.D. degree or the equivalent, and should ordinarily be not more than thirty-five years of age. There are no limitations with respect to citizenship of applicants.

SYMPOSIUM INTERNAZIONALE SUI FARMACI PSICOTROPI

At the beginning of May, 1957, Professor E. Trabucchi will present an international Symposium on the "psicotropic substances," and their pharmacological and clinical aspects, in relation to their employment in psychiatric therapy.

All particular information can be obtained on request from the secretary, c/o Institute of Pharmacology, University of Milano, Via Andrea del Sarto, 21, Milano, Italy.

ERRATA

In the note by N. S. Halmi and R. G. Stuelke appearing in the July 1956 issue of *Endocrinology* the following changes should be noted:

Page 135, Table 1, penultimate line, 45 hours, should read 44 hours.

Page 136, Table 2, top caption line should read 20 mg. PTU/day for 9 days instead of 20 mg. PTU/day for 0 days.